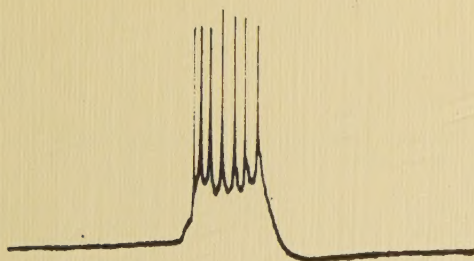




From the Institute of Physiology and Biophysics
University of Lund
Sweden

**EFFECTS OF IONS, OSMOLALITY, PASSIVE STRETCH
AND OXYGEN ON ELECTRICAL AND MECHANICAL
ACTIVITY IN VASCULAR SMOOTH MUSCLE**



BY

STEFAN B. SIGURDSSON

LUND 1980

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AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska
fakulteten vid Lunds Universitet för avläggande
av filosofie doktorsexamen kommer att offentligen
försvaras å Fysiologiska institutionens stora före-
läsningssal fredagen den 13 juni 1980 kl. 9.15.

av

Stefan B. Sigurdsson

B.Sc. Vrml.

Avhandlingen kommer att försvaras på svenska språket

Lund 1980

Organization
LUND UNIVERSITY

Institute of Physiology and Biophysics,

Sölvegatan 19

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Document name

DOCTORAL DISSERTATION

Date of issue

800613

CODEN:

Sponsoring organization

Title and subtitle

Effects of ions, osmolality, passive stretch and oxygen on electrical and mechanical activity in vascular smooth muscle.

Abstract

The electrical and mechanical responses of the isolated rat portal vein to ionic changes and variations in osmolality, passive stretch and P_{O_2} were examined. Extracellular Ca^{2+} is required to trigger the release of superficially bound Ca^{2+} causing the fast phase in the response to K^+ -depolarization. The extracellular Ca^{2+} is depleted in about 3 min and the superficial pools are emptied during the first 30 min in Ca^{2+} -free solution. The slow phase of the contracture obtained after depletion of the superficial pools uses mainly extracellular Ca^{2+} . Sr^{2+} and Ba^{2+} are able to substitute for Ca^{2+} in spontaneous electrical activity but excitation-contraction coupling is less effective when Ca^{2+} is replaced by Sr^{2+} or Ba^{2+} . The mechanical responses in Sr^{2+} and Ba^{2+} solutions appear to be combined effects of liberation of tissue bound Ca^{2+} and a direct action. Mg^{2+} seems not able to substitute for Ca^{2+} in the spontaneous electrical and mechanical activity. Instead, Mg^{2+} depresses the electrical excitability of the cell membrane and even interferes with intercellular propagation. A rate dependent response to stretch and shortening was observed in isolated vascular smooth muscle. This response was similar under isometric and isotonic conditions. The excitatory response to dynamic stretch is more closely related to dP/dt than to dL/dt . Hyperosmolality inhibited and hypoosmolality excited the spontaneous electrical and mechanical activity. These responses faded during prolonged exposure. The "mechanical effectiveness" of the spikes decreased during prolonged exposure to hypoosmolality and increased in hyperosmolality. The electrical and mechanical activity was abolished in severe hypoxia ($P_{O_2} < 5$ mm Hg). The activity could be reinitiated during hypoxia by various stimulating agents and by increase in $[K^+]$ and decrease in $[Na^+]$. The uptake and efflux of $^{42}K^+$ seemed not to be affected directly by hypoxia. Hypoxia did not change the membrane potential significantly. Moderate hypoxia ($P_{O_2} \leq 10$ mm Hg) inhibited the mechanical activity more than the electrical activity. It is suggested that hypoxia exerts its inhibitory action through a combined effect of shortage of substrate, depressed electrical activity and an electro-mechanical uncoupling which is possibly a pH dependent effect.

Key words

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language

English

ISSN and key title

ISBN

Recipient's notes

Number of pages

155

Price

Security classification

Distribution by (name and address)

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LUND 1980

This thesis is based on the following original papers which will be referred to in the present text by their Roman numerals.

- I Sigurdsson S.B., Uvelius B. and Johansson B. 1975. Relative contribution of superficially bound and extracellular calcium on activation of contraction in isolated rat portal vein. *Acta Physiol Scand* 95:263-269.
- II Uvelius B., Sigurdsson S.B. and Johansson B. 1974. Strontium and barium as substitutes for calcium on electrical and mechanical activity in rat portal vein. *Blood Vessels* 11:245-259.
- III Sigurdsson S.B. and Uvelius B. 1977. The effects of variations in extracellular magnesium concentration on electrical and mechanical activity in rat portal vein. *Acta Physiol Scand* 99:368-376.
- IV Sigurdsson S.B., Johansson B. and Mellander S. 1977. Rate-dependent myogenic response of vascular smooth muscle during imposed changes in length and force. *Acta Physiol Scand* 99:183-189.
- V Sigurdsson S.B. and Johansson B. 1980. Quantitative aspects of electrical and mechanical responses to anisomolar solutions in the smooth muscle of the rat portal vein. *Acta Physiol Scand*. In press.
- VI Sigurdsson S.B., Orlov R., Hellstrand P. and Johansson B. 1980. Responses to ions and vasoconstrictor agents and changes of potassium fluxes in vascular smooth muscle during hypoxia. *Acta Physiol Scand*. Submitted for publication.
- VII Sigurdsson S.B. and Grampp W. 1980. The effect of hypoxia on mechanical and electrical properties of smooth muscle from the rat portal vein. Manuscript.



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1. GENERAL INTRODUCTION

The membrane potential caused by the ionic gradients across the semi-permeable cell membrane, the action potential caused by changes in the permeability characteristics of the membrane, and the relationship of these mechanisms to the contractile activity have been intensively studied in the vascular smooth muscle cells during the last few decades. Knowledge of these mechanisms is of great importance for the general understanding of the control of the peripheral circulation. In the resistance and precapillary sphincter vessels, stimulatory and inhibitory responses can be initiated by variations in the transmural pressure and by changes in the environment with respect to chemical factors such as ions, metabolites, oxygen etc. These factors have been investigated intensively *in vivo* but experiments on isolated vessels have also been very useful to clarify at least some of the cellular mechanisms operating to control the activity in vascular smooth muscle (for ref see Mellander and Johansson, 1968; Jonsson, 1970; Lundvall, 1972; Weiss, 1977; Hellstrand, 1979; Johansson and Somlyo, 1980).

The ion mechanisms which lie behind the membrane potential, the action potential and the contractile activity are today relatively well known in skeletal muscle, less so in heart muscle and still rather speculative in smooth muscle. The Ca^{2+} ion plays an essential role in all three types of muscle but its relative importance in the different cellular processes varies. In skeletal muscle Ca^{2+} is physiologically important in the control of the contractile mechanism (Ebashi and Endo, 1968), in heart muscle there is also a Ca^{2+} dependent inward current connected with the contractile mechanism, directly or indirectly (Fozzard, 1977). In smooth muscle Ca^{2+} seems to be the main ion in the whole excitation-contraction mechanism. Knowledge of the role of Ca^{2+} in this tissue is therefore of fundamental importance.

The aim of the studies presented here was to elucidate further the role of Ca^{2+} and other divalent ions in the electro-mechanical coupling of isolated vascular smooth muscle preparations and to investigate the effects on this process of several local factors such as oxygen, osmolality and change in length.

2. EFFECTS OF DIVALENT IONS

2.1 Introduction

The cells of smooth muscle as well as of striated muscle contain thick and thin myofilaments which are constituted from the proteins myosin and actin respectively. These proteins are considered to interact with each other for producing tension in both types of muscle and the activity of the myofibrillar ATP-ase supplies energy to this contractile machinery. In skeletal muscle the regulatory proteins troponin and tropomyosin cause inhibition of the ATP-ase activity in the relaxed state of the muscles by suppressing the interaction between actin and myosin. The inhibitory action of these regulatory proteins is maintained at low intracellular Ca^{2+} concentration; in the relaxed muscle the concentration of Ca^{2+} seems to be below 10^{-7}M . If the concentration is increased to above this threshold the inhibitory effect of the troponin-tropomyosin system is abolished and it is possible for myosin to interact with actin sites and contraction is initiated. The maximal contraction is reached when $[\text{Ca}^{2+}]_i$ is $10^{-5} - 10^{-4}\text{M}$. (Ebashi and Endo, 1968, Huxley, 1974).

In smooth muscle tension generation by the contractile proteins is also considered to be triggered by a rise in $[\text{Ca}^{2+}]_i$, but the regulatory system appears to be different. It has been indicated recently that calcium may act on regulatory mechanisms involving phosphorylation of a myosin light chain (Small and Sobieszek, 1977). Other types of regulatory proteins needed for contraction in smooth muscle have also been described (Ebashi, 1978).

In skeletal muscle the structure regulating the intracellular concentration of Ca^{2+} is the sarcoplasmatic reticulum from which Ca^{2+} is released for contraction and stored during relaxation (Huxley, 1974). In smooth muscle the source of the activator Ca^{2+} is uncertain. There is evidence that extracellular Ca^{2+} may enter during the action potential as the main ion carrying the inward current (Bulbring and Tomita, 1970). This influx of Ca^{2+} ions may contribute to the activation of the contractile system, but release from binding sites in the membrane

or intracellular stores is another possible mechanism. A decrease in $[Ca^{2+}]_i$, seems to be necessary for relaxation in smooth muscle. The mechanism may be an uptake into the sarcoplasmatic reticulum or other intracellular depots and/or extrusion of Ca^{2+} out of the cell (Devine et al., 1973; Reuter et al., 1973; van Bremen, 1976)

The Ca^{2+} ion, besides the action described above, also has a membrane stabilizing effect i.e. the higher the extracellular $[Ca^{2+}]$ the less excitable the membrane (Bohr, 1978). The fact that the Ca^{2+} ions are involved in several different processes affecting the mechanical output of the cell makes it difficult to study the individual steps in the excitation-contraction coupling. Otherdivalent ions such as Sr^{2+} , Ba^{2+} and Mg^{2+} may be able to substitute for Ca^{2+} in some of these different processes (Bulbring and Tomita, 1970). Recent studies have shown that vessels from normotensive and hypertensive rats have different reactivity to Sr^{2+} and Ba^{2+} and that this difference might be related to alteration in the calcium binding properties in hypertension (Bohr, 1974; Wei et al., 1977). More knowledge of the role of Ca^{2+} and of the effects of Sr^{2+} and Ba^{2+} on normal vascular smooth muscle is therefore of interest. The purpose of the studies reviewed in this section was to investigate the source of activator Ca^{2+} and the effect of other divalent cations on the electrical and mechanical events in vascular smooth muscle.

2.2 Outline of methods

The preparation used in the present expts was the isolated rat portal vein. This preparation was chosen because its myogenic activity may make it a good model of the circulatory more interesting microvessels in the peripheral vascular bed (Ljung, 1970). The preparations were mounted in two different ways. For experiments where only isometric tension was studied, about 5 mm of the vessel was mounted in a mantled organ bath between a muscle holder and a transducer. The experiments with simultaneous recording of electrical and mechanical activity were carried out on 15-20 mm sections of the portal mesenteric vein using either the sucrose gap apparatus or a set of three extracellular pipettes. All preparations were kept at a preload of 4 mN.

The bathing medium was a Tris buffered solution. The reason for using Tris was that the divalent cations at higher concentrations precipitate in bicarbonate buffered Krebs solution. The isometric force was quantitated by integrating the active tension curve with an electronic integrator device and the electrical signals were recorded in a DC mode. To obtain solutions with extremely low Ca^{2+} or Mg^{2+} , 1 mM EGTA or EDTA respectively, was added to nominally Ca^{2+} or Mg^{2+} free solutions. The EGTA solution lowered $[\text{Ca}^{2+}]_0$ and $[\text{Mg}^{2+}]_0$ to $< 10^{-9}\text{M}$ and $< 10^{-8}\text{M}$ respectively (Hubbard, 1958). K-high solutions used to depolarize the preparation were obtained by replacing all NaCl with equimolar amounts of KCl.

2.3 Results and comments

2.3.1 Ca^{2+} and its superficial binding sites (Paper I).

During the accommodation period in Tris solution with 2.5 mM Ca^{2+} the preparation showed regular contractions each initiated by a burst of spikes. When the preparation was exposed to a Ca^{2+} -free solution the spontaneous activity decreased and disappeared in about 3 min. This implies that the extracellular $[\text{Ca}^{2+}]$ in the tissue is rapidly reduced in the nominally Ca^{2+} free medium. This was further supported by results from experiments with depolarizing solutions. If the preparation was exposed to Ca^{2+} -free K⁺-high solution (120 mM) after 5-6 min in the Ca^{2+} -free medium, no electrical spikes or mechanical activity (Fig.1c) were observed. Previous studies have shown that the threshold concentration is in the range of 0.1 - 0.3 mM Ca^{2+} for both contracture and spontaneous activity (Biamino and Johansson, 1970).

If the muscle was depolarized after 5-6 min in Ca^{2+} -free medium with a solution containing 120 mM K⁺ and 2.5 mM Ca^{2+} , a contracture developed with few electrical spikes occurring during the rising phase. The rate of rise of the contracture was found to depend on the duration of the exposure of the muscle to the Ca^{2+} -free solution as shown in Fig.1a and b. Increasing the duration in the Ca^{2+} depleting solution stepwise from 6 to 30 min gradually reduced the fast phase of the contracture and the spike activity was abolished. A further increase in the Ca^{2+} washout time

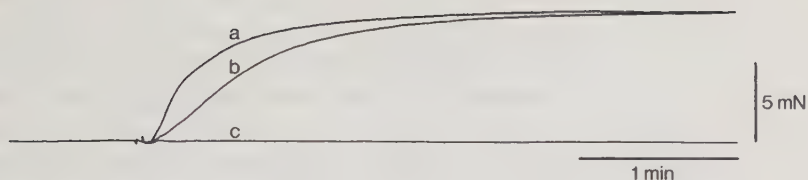


Fig. 1 Mechanical responses to K^+ -Tris 2.5 mM Ca^{2+} after exposure to Ca -free solution for 6 min (a) and 30 min (b). The response to K -Tris 0 mM Ca after 6 min in Ca -free Tris (c).

did not have any additional effects as the shape of the responses after 30 and 60 min was identical.

These results indicate that the extracellular or loosely bound Ca^{2+} was washed out in 5-6 min, as the spontaneous activity and the response to depolarizing solution disappeared within this time. The change in response to K -high solution with 2.5 mM Ca^{2+} over the 6 to 30 min period is therefore not supposed to be caused by a difference in the amount of Ca^{2+} remaining extracellularly, but to general depletion of a superficially bound fraction of Ca^{2+} which can be mobilized and contribute to the contractile activation. A certain amount of $[Ca^{2+}]_o$ seemed to be required to trigger the release of this bound fraction, and after 5-6 min depletion the extracellular Ca^{2+} had dropped below this level. Therefore simultaneous administration of Ca^{2+} with the depolarizing solution was required when the preceding exposure to Ca^{2+} -free medium had lasted more than 5-6 min.

The superficial " Ca^{2+} -pools" seem to be emptied after about 30 min of Ca^{2+} depletion, and the K^+ response elicited under these conditions depends almost exclusively on influx of extracellular Ca^{2+} . The evidence for this is that the response to K -high solution does not change further with prolonged exposure to a Ca^{2+} -free solution and that the amplitude of this response can be graded directly by variation in $[Ca^{2+}]_o$ (c.f. Biamino and Johansson, 1970; Hellstrand, Johansson and Ringberg, 1972)

The amounts of Ca^{2+} which can be released from the different Ca^{2+} pools are, according to our expts, not only dependent on the time in the preceding Ca^{2+} -free solution but also on the access of the muscle cells to Ca^{2+} during the accommodation period. The pools seem practically saturated in solutions with a normal level of Ca^{2+} (2.5 mM) as increasing the Ca^{2+} in the accommodation period to 5 mM gave no change in the response to K-high solution with 2.5 mM Ca^{2+} after 5-6 min in Ca^{2+} -free media. If however the Ca^{2+} concentration in the accommodation solution was lowered below 2.5 mM a slower rise of the contracture elicited after 5-6 min in a Ca^{2+} -free solution was obtained indicating incomplete saturation of the pools.

Even when the cells are in the depolarized state they seem able to accumulate Ca^{2+} into the pools. If the time in Ca^{2+} -free solution between two K^+ contractures (of 4 min duration each) is less than 10 min the later contracture seems to be able to use Ca^{2+} accumulated during the previous contracture. This is seen by the rate of rise of the later contracture which increases if the time in Ca^{2+} depleting solution is less than 10 min.

2.3.2 Sr^{2+} and Ba^{2+} as substitutes for Ca^{2+} (Paper II)

Experiments were done to study the effects of graded concentrations of Ca^{2+} , Sr^{2+} and Ba^{2+} on the spontaneous electrical and mechanical activity and the results are exemplified by original recordings from three experiments shown in Fig.2. In $A_1 - A_5$ the effect of increases in $[\text{Ca}^{2+}]_0$ from 0 to 0.25, 0.5, 2.5 and 10 mM are shown. In $B_1 - B_5$ and $C_1 - C_5$ are shown the effects of similar concentrations of Ba^{2+} and Sr^{2+} respectively. A_1 , B_1 and C_1 show that all electrical and mechanical activity was abolished in Ca^{2+} , Ba^{2+} and Sr^{2+} -free solutions. In 0.25 mM (A_2 , B_2 and C_2) solutions activity appeared only in Ba^{2+} , where small rhythmical electrical spikes were observed associated with small contractions. If the concentrations of the divalent ions were increased to 0.5 mM spontaneous electrical and mechanical activity appeared in all preparations. In the Ba^{2+} solution the mechanical activity was of tetanical type instead of the phasic pattern seen in Ca^{2+} and Sr^{2+} solutions. However, the activity in Sr^{2+} solution was still very small

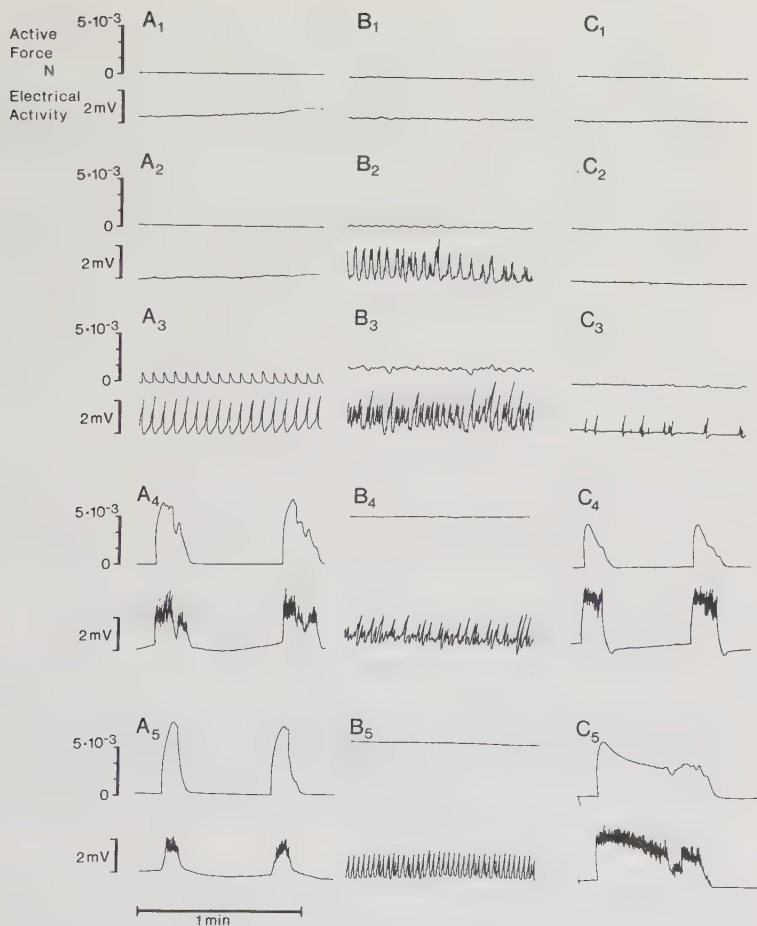


Fig. 2 Electrical and mechanical activity of the portal vein at different levels of $[Ca^{2+}]_o$ (A), $[Ba^{2+}]_o$ (B) and $[Sr^{2+}]_o$ (C). In A₁ - A₅, B₁ - B₅ and C₁ - C₅ are shown the effects of adding 0 - 0.25 - 0.5 - 2.5 and 10 mM of respective ion after 30 min in Ca-free tris solution.

at 0.5 mM. In 2.5 mM solutions regular phasic contractions with a frequency of about 2 per min associated with bursts of spikes were seen with both Ca^{2+} and Sr^{2+} . In Ba^{2+} a tetanic contraction with continuous spiking was seen. When the concentration of the ions was increased to 10 mM, the phasic activity was decreased in Ca^{2+} solution, tetanic contracture with continuous spiking still persisted in the Ba^{2+} solution, and long phasic contractions associated with long bursts of spikes appeared in Sr^{2+} . The frequency of the contractions in the Sr^{2+} solution was only 0.5 per min. The different types of activity described above could be maintained for hours in Ca^{2+} and Sr^{2+} solutions, but during prolonged exposure to Ba^{2+} the tetanic response decreased with time even though the electrical spike discharge was almost unchanged.

An integration of the isometric force developed in the Ca^{2+} , Sr^{2+} and Ba^{2+} solutions was performed to obtain a quantitative comparison between the contractile effects of the three divalent cations. The muscles were exposed for 10 min to increasing concentrations of the respective ion, each exposure being preceded by a 15 min period of relaxation in nominally Ca^{2+} -free solution. The concentration response curve for each of the ions is shown in Fig.3. For Ba^{2+} the maximum occurs at 2 mM, for Ca^{2+} at 4 mM and for Sr^{2+} at about 16 mM. The mechanical response decreases at the highest concentrations of Ca^{2+} and Ba^{2+} . With regard to Ca^{2+} this decrease can be attributed to the change in electrical activity (Fig.2A). In the case of Ba^{2+} it may be related to the time-dependent decline in tetanic force mentioned above.

The amplitudes of the K-contractures in solutions with increasing amounts of the divalent ions were also compared. The concentrations for the maximal responses were found to be similar to those given above for integrated spontaneous activity and the curve for Ba^{2+} decreased at the higher concentrations. This observed difference between the concentrations of the 3 ions required for maximal response was reduced if the muscles were accommodated in solution with 2.5 mM Ca^{2+} before each exposure to the different Ca^{2+} , Ba^{2+} or Sr^{2+} concentrations. The maximal response was then obtained at about 2.5 - 5 mM for each ion and the decrease in the Ba^{2+} curve at the higher concentrations had disappeared which indicates

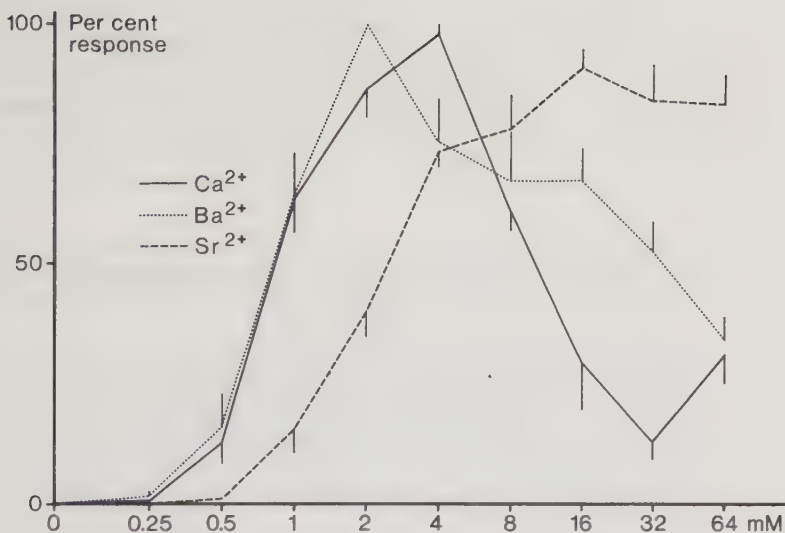


Fig.3 Concentration-effect curves for Ca^{2+} , Sr^{2+} and Ba^{2+} with regard to integrated active force (spontaneous activity) in this solution. All values expressed in per cent of the maximum obtained in each preparation with the respective ion (mean $\pm$ S.E., $n = 4$ for each ion).

that Sr^{2+} and Ba^{2+} were more effective when some Ca^{2+} was present. In order to test further if Ca^{2+} was so important for the action of Sr^{2+} and Ba^{2+} the effect of Ca^{2+} depletion was studied on contractures. After a control contracture, the preparations were placed for various times in Ca^{2+} -free solution with 1 mM EGTA before a new contracture was elicited. The relative contracture tension obtained for each ion as expressed in per cent of the respective control amplitude is shown in Fig.4. After about 30 min in the Ca^{2+} -free EGTA solution the mean

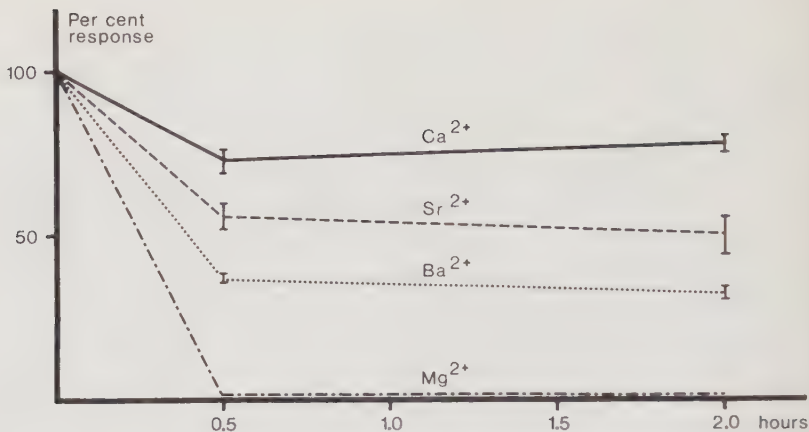


Fig. 4 Amplitude of potassium contractures obtained with 2.5 mM Ca^{2+} , Sr^{2+} , Ba^{2+} , and Mg^{2+} after 0.5 or 2 h in Ca -free solution with 1 mM EGTA. Mean ($\pm$ S.E.) expressed in percent of control contractures produced by respective ion (2.5 mM) before exposure to the Ca -free solution. The control contractures with Sr^{2+} , Ba^{2+} , and Mg^{2+} were $81 \pm 2\%$, $97 \pm 2\%$ and $63 \pm 3\%$ respectively of corresponding contractures in solution with 2.5 mM Ca^{2+} ($n=12$ for each ion).

contracture force developed in K^+ -high solutions with Ca^{2+} , Sr^{2+} or Ba^{2+} (2.5 mM) were about 75, 55, and 35 per cent of their controls. No further decrease occurred after 2 h in the Ca^{2+} -free EGTA solution. These results indicate that some of the mechanical effects of Sr^{2+} and especially Ba^{2+} in K^+ -high solution are dependent on release of tissue bound Ca^{2+} .

The divalent ions seem to be able to elicit spike activity even in Na^+ -free solutions. The evidence for this was obtained in experiments where the muscle had been placed in Na^+ and Ca^{2+} -free solutions for at least 30 min. When small amounts (0.4 mM) of Ca^{2+} , Sr^{2+} , or Ba^{2+} were added to the solution small electrical spikes appeared. It therefore seems reasonable to suggest that the above membrane effects of Sr^{2+} and Ba^{2+} are direct and not mediated through release of bound Ca^{2+} .

2.3.3 The effects of Mg^{2+} (Paper III)

The effects of variations in extracellular Mg^{2+} concentration on the spontaneous activity are shown in Fig. 5. All the recordings were obtained from the same preparation. Maximal integrated active force was obtained in Mg^{2+} -free solutions and stepwise increase in $[Mg^{2+}]_o$ decreased the mechanical output. There was a decrease in contraction

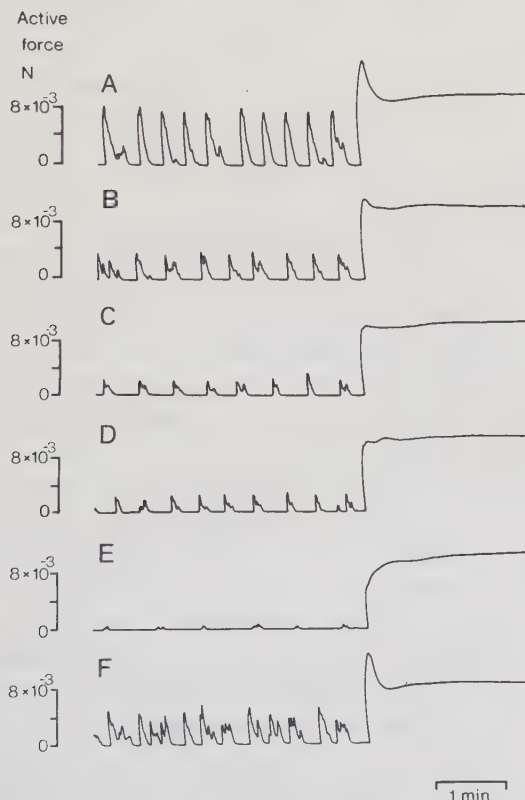


Fig. 5 In the left half is shown the effect on the spontaneous activity of one preparation by increasing the $[Mg^{2+}]_o$ stepwise from 0 (A) to 1.2 (B), 2.4 (C), 4.8 (D), and 10 mM (E). In (F) is shown how the muscle recovers in solution with 0 mM Mg^{2+} . In the right half are shown potassium contractures in the respective solutions.

amplitude and in frequency of contractions. In solution with 10 mM Mg^{2+} only minor contractions were visible. In sucrose gap experiments where the electrical and mechanical activity were recorded simultaneously spike activity appeared to decrease in proportion to the decrease in mechanical activity when Mg^{2+} was increased. By increasing $[K^+]_o$ from 6 to 12 mM in solutions with the higher Mg^{2+} concentrations it was possible to almost restore the normal pattern of spontaneous activity present in standard solution. Therefore the response to increased $[Mg^{2+}]_o$ can be explained largely as a hyperpolarizing effect of Mg^{2+} .

The effect of extracellular Mg^{2+} on K^+ -contractures (120 mM K^+) are shown in the right half of Fig.5. The phasic part of the response was decreased in a similar way as the spontaneous activity when $[Mg^{2+}]_o$ was increased, but the sustained contracture amplitude was almost unaltered. A quantitation of the Mg^{2+} effect on the spontaneous activity (full lines) and on K^+ -contractures (broken lines) at different $[Ca^{2+}]_o$ is shown in Fig.6. For all $[Ca^{2+}]_o$ studied the integrated spontaneous mechanical activity decreased rapidly when $[Mg^{2+}]_o$ was increased. The amplitude of the sustained K^+ -contractures was unaffected by changes in $[Mg^{2+}]_o$ when $[Ca^{2+}]_o$ was above 0.5 mM. In solutions with $[Ca^{2+}]_o \leq 0.5$ mM a decrease in response was seen for the higher $[Mg^{2+}]_o$. These results show that the smaller the $[Ca^{2+}]_o$ is the more sensitive will the muscle be to changes in $[Mg^{2+}]_o$.

It can therefore be concluded that Mg^{2+} cannot replace Ca^{2+} for the electrical and mechanical activity. These results also indicate that Mg^{2+} cannot trigger the release of Ca^{2+} from the intracellular binding sites. This inability of Mg^{2+} to replace Ca^{2+} is also clearly seen in Fig.4 in that no contracture could be elicited in solutions where Ca^{2+} was replaced by equimolar amounts of Mg^{2+} .

By placing three extracellular glass capillary electrodes on different places on the preparation it was possible to obtain information on how increased Mg^{2+} affected propagation of the electrical activity along the muscle. In a normal solution (1.2 mM Mg^{2+}), the areas under the three electrodes were all activated and the spike bursts recorded by each of

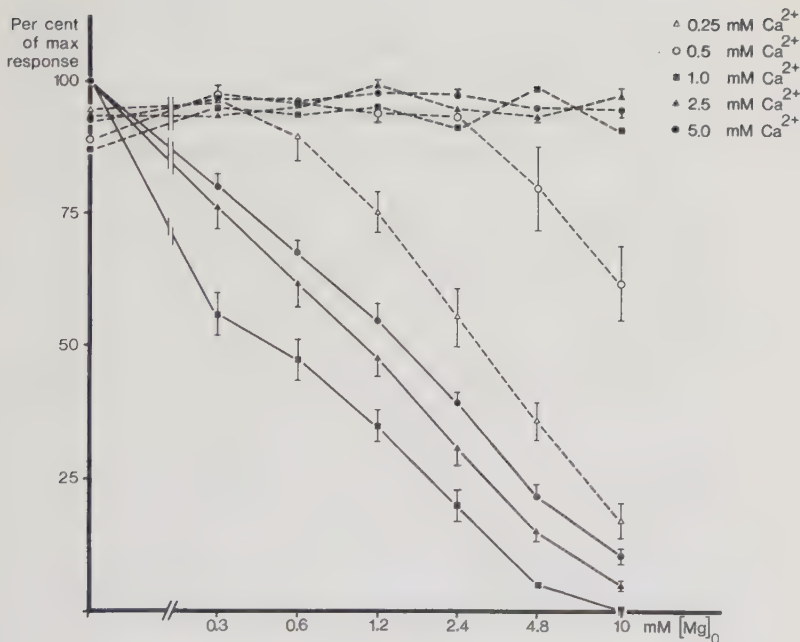


Fig.6 Quantitative effects of stepwise increase in $[Mg^{2+}]_0$ from 0 to 10 mM in solutions with various $[Ca^{2+}]_0$ expressed as percentages of the maximal force. Broken lines show the effects on potassium contractures, full lines effects on integrated spontaneous activity. Means $\pm$ S.E., $n=5$.

them were similar in size and duration. When extracellular Mg^{2+} was increased there was a tendency towards desynchronisation of the electrical and mechanical activity and propagation was impaired. The number of spikes in each burst often decreased as it propagated along the muscle and some bursts failed to activate the area under the electrode most remote from the "pacemaker area".

Prolonged exposure to Mg^{2+} -free solutions did not affect the increased contractile activity of the preparation. However, if the muscles were

placed in Ca^{2+} and Mg^{2+} -free solution with 1 mM EDTA for 1 h the activity rapidly disappeared, no spontaneous activity occurred when Ca^{2+} (2.5 mM) was added to the solution. However, very small contractures (10% of control) could still be elicited in Mg^{2+} -free, K^+ -high solutions with 2.5 mM Ca^{2+} . If Mg^{2+} (1.2 mM) was readministered spontaneous activity reappeared and contractures of almost normal amplitude could be obtained.

2.4 Discussion

The role of the different cations will be discussed below with respect to membrane function, electro-mechanical coupling, contraction and relaxation.

Our results showed that the spontaneous electrical and mechanical activity of the portal vein was abolished within 2-3 min in Ca^{2+} -free solution. If the solution was both Na^+ and Ca^{2+} -free it was possible to obtain spike activity when small amounts of Ca^{2+} were readministered. Sr^{2+} and Ba^{2+} but not Mg^{2+} , were able to replace Ca^{2+} and elicit spikes in Na^+ -free solutions. The former ions also restored electrical and mechanical activity after incubation in Ca^{2+} -free solution with EGTA. These results indicate that Ca^{2+} can function as the charge carrier for the inward current at least under these conditions. They also indicate that Sr^{2+} and Ba^{2+} can substitute for Ca^{2+} directly at the membrane level.

The ionic mechanisms of the action potential in portal vein therefore are quite different in many important respects from those in nerve and skeletal muscle. Further evidence in this direction is given by the fact that the action potentials in the smooth muscle of the portal vein are insensitive to tetrodotoxin (Ito and Kuriyama, 1971) and that they are blocked instead by Mn^{2+} and so called Ca^{2+} -antagonistic drugs (Golenhofen et al., 1973). However, the role of Ca^{2+} under conditions where Na^+ is also available is still an open question, because even though no spikes are elicited in Ca^{2+} -free solution this does not exclude the possibility of an inward Na^+ current under normal conditions. Keatinge, (1968) concluded from his experiments on sheep carotid arteries that Na^+ is the principal ion carrying the inward current of the action potential in this preparation. This is perhaps a good example of how the relative role of Ca^{2+} and Na^+ currents in the action potential varies between

different smooth muscle types. Anyway Ca^{2+} seems to be the important ion for spike generation in the portal vein.

In a study on myocardial fibers Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} have all been found to utilize the same membrane channels and act as a charge carrier for the slow inward current (Kohlhardt et al., 1973). This is probably valid for Ca^{2+} , Sr^{2+} and Ba^{2+} in our preparation but apparently not for Mg^{2+} . Increased Mg^{2+} caused inhibition of both electrical and mechanical activity perhaps partly through a hyperpolarization of the membrane as depolarization by a moderate increase in K^{+} restored the activity. This inability of Mg^{2+} to substitute for Ca^{2+} may be caused by its larger diameter (8Å) compared to the other divalent ions (5-6Å) (Harris, 1960) making its passage through the membrane channels difficult.

There is little doubt that action potentials when they occur in vascular smooth muscle are involved in the normal triggering of contraction. However, it is also clear that the electrical events do not influence the contractile system directly. The extent of the contraction seems to be regulated by variation in intracellular Ca^{2+} between 10^{-7} and 10^{-5}M , as indicated by studies of isolated vascular actomyosin (Sparrow et al., 1970; Litten et al., 1977) and by studies on chemically skinned or glycerinated smooth muscle (Endo et al., 1977; Gordon, 1978).

The mechanism by which the spike produces increase in $[\text{Ca}^{2+}]_i$ is still a matter of debate. The influx of Ca^{2+} via the spike discharges seems not to be enough. Calculations by Johansson and Somlyo (1980) have shown that even if the entire charge in an action potential were carried by Ca^{2+} it would be insufficient to account for the activation of contraction. Therefore it has been suggested that the influx of Ca^{2+} can cause a release of Ca^{2+} from superficial or intracellular bound fractions (Ca^{2+} pools) that raises $[\text{Ca}^{2+}]_i$ sufficiently to initiate contraction. The divalent ions Sr^{2+} and Ba^{2+} appear to substitute for Ca^{2+} at this level and trigger the release of Ca^{2+} from these pools, but they seem not as effective as Ca^{2+} itself. The mechanical output elicited by the spikes in Ca^{2+} solution was larger than the corresponding response to Sr^{2+} and

Ba^{2+} . This is also clearly seen in Fig. 3. For Ba^{2+} the low effectiveness in coupling is compensated by its excitatory action at the membrane so that the integrated force becomes relatively large (see also Fig. 1, B_1-B_5). For Sr^{2+} ineffective coupling shifts the concentration-response curve to the right relative to that for Ca^{2+} .

After five to six min of Ca^{2+} depletion the spontaneous activity is abolished and no contractile responses to depolarization of the muscle with Ca^{2+} -free and K^+ -high solution are observed. If Ca^{2+} is added simultaneously with the depolarizing solution a superficially bound Ca^{2+} fraction seems to be mobilized and it contributes to the contractile activation and to the spike phenomenon which after short periods in Ca -free solution accompanies the first, fast phase of the contracture (Fig. 1). If the muscle was exposed for longer periods (6-30 min) to the Ca^{2+} depleting solution the spike phenomenon disappeared rapidly and the fast phase decreased gradually with time.

The shapes of the responses after more than 30 min depletion remained constant with respect to rate of rise and amplitude and this stage is therefore suggested to define the slow contracture phase which appears markedly to depend on influx of Ca^{2+} from the outside. The fact that tonic contracture can be maintained in depolarizing K^+ -high solution in normally spontaneously active preparations as the portal vein shows that the action potentials are not at all necessary for eliciting the contraction.

The possibility of different sources of activator Ca^{2+} has been discussed above. Collins, Sutter and Teiser (1972a) found two components in the response to noradrenaline and potassium in the rabbit anterior-mesenteric portal vein. They suggested that the early phase was related to liberation of superficially bound Ca^{2+} and the later on influx of Ca^{2+} from the outside. Further evidence has been presented by several authors that different stimuli can differ in their relative utilization of different Ca^{2+} pools (Peiper et al., 1971; van Bremen et al., 1972; Golenhofen et al., 1973; Droogmans et al., 1977). In our study Sr^{2+} seems to be able to replace Ca^{2+} to some extent at the level of the

contractile mechanism, but Ba^{2+} ions seem to mediate their effects mainly via release of Ca^{2+} from binding sites. This effect of Ba^{2+} is clearly shown by a long time exposure to Ba^{2+} solution as there is a decrease with time of the tetanic tension, even though the spike activity is continuous. This intensive spike activity should increase Ca^{2+} in the beginning and cause the strong contraction but the decrease in force with time could then depend on loss of Ca^{2+} to the extracellular media. The experiments shown in Fig.4 indicate however that Ba^{2+} and especially Sr^{2+} can to a certain degree act directly at the contractile level. (See also Hudgin and Weiss, 1969; Weiss, 1977).

In a metabolically active vascular smooth muscle $[\text{Mg}^{2+}]_i$ is considered to be maintained at a level of about 10^{-4}M (Palaty, 1974). This concentration seems to remain relatively constant due to an active Mg^{2+} extrusion mechanism as the extracellular Mg^{2+} concentration is much higher ($1.2 \times 10^{-3}\text{M}$). $[\text{Mg}^{2+}]_i$ can only be lowered in Mg^{2+} -free Ca^{2+} -free solution because low external Ca^{2+} markedly increases the membrane permeability to Mg^{2+} (Palaty, 1974). After about 1 h in Mg^{2+} -free Ca^{2+} -free solution with 1 mM EDTA, which lowers $[\text{Mg}^{2+}]_o$ to $< 10^{-8}\text{M}$ (Hubbard, 1958), all contractile activity disappeared in our expts. Even if $[\text{Ca}^{2+}]_o$ was normalized at this stage (2.5 mM) no spontaneous activity and only very small K^+ -contractures were obtained. Adding Mg^{2+} (1.2 mM) restored both kinds of responses. These results indicate that the effects of Mg^{2+} depletion are not exerted at the level of the electromechanical coupling but involve the function of the contractile proteins themselves. The optimal activity for the Mg^{2+} -ATP-ase in arterial smooth muscle is obtained with a free $[\text{Mg}^{2+}]$ of about 10^{-4}M . (Murphy et al., 1969).

Even though it seems possible to inhibit the contractile activity by lowering $[\text{Mg}^{2+}]_i$, such a mechanism is unlikely to be involved in physiological mechanisms of vasodilation (Paul and Rüegg, 1978).

For relaxation, the concentration of the divalent ions participating in the regulation of the contractile force has to be reduced. This

reduction is believed to be brought about by extrusion or sequestration of the ions. In a recent study (Uvelius and Sigurdsson, 1980) on the rate of relaxation from K^+ -contractures it was found that the time to 50% relaxation of the initial tension was similar for contractures elicited in 2.5 mM Ca^{2+} , Sr^{2+} or Ba^{2+} solutions. This indicates that some sequestration and/or extrusion mechanism exists, unspecific enough to lower the concentration of all the three ions to subthreshold levels. Somlyo and Somlyo and their collaborators have shown in their studies that sarcoplasmic reticulum in vascular smooth muscle is able to accumulate Ca^{2+} in high concentration (Somlyo et al., 1979) and to bind Sr^{2+} ions (Somlyo and Somlyo, 1971). They also found that mitochondria accumulate Ca^{2+} , Sr^{2+} and Ba^{2+} (Somlyo et al., 1974). Johansson and Somlyo (1980) point out in a recent review that intracellular uptake of Ca^{2+} rather than efflux appears as the major mechanism for relaxation which continues even at an accelerated rate when the Ca^{2+} efflux is blocked with lanthanum.

A summarizing diagram of the results discussed here is shown in fig. 7. Of the divalent ions (A) Ca^{2+} , Sr^{2+} , and Ba^{2+} but not Mg^{2+} can be charge carriers for the action potential inward current. Ca^{2+} and Sr^{2+} seem to exert some and Ba^{2+} most of their effects on the contractile proteins (C) via release of tissue bound Ca^{2+} (B). The sequestration and extrusion mechanisms are unspecific enough to handle Ba^{2+} , Sr^{2+} as well as Ca^{2+} (D).

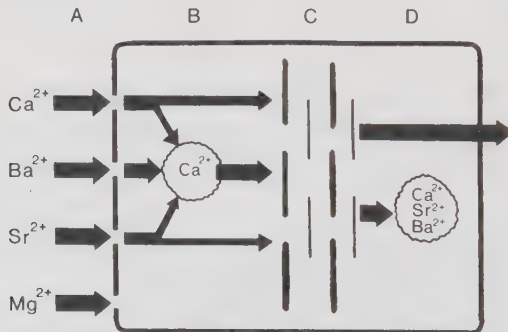


Fig. 7. Schematic illustration showing proposed effects of Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} on cell membrane (A) tissue bound Ca^{2+} (B), the contractile proteins (C) and reuptake and extrusion of ions (D).

3. EFFECTS OF OSMOLALITY AND OF CHANGES IN LENGTH

3.1 Introduction

The role of local mechanisms in the control of the peripheral circulation is to establish an optimal exchange function in the tissue. Two of the main factors proposed to be responsible for local control are changes in the chemical metabolic environment in the tissue and myogenic smooth muscle reactions related to stretch.

Variation in metabolic activity causes a number of specific changes in the composition of the interstitial fluid but there will also be an unspecific change in osmolality which, in its own right, will influence the smooth muscle tone of the resistance vessels (Mellander and Johansson, 1968; Lundvall, 1972). If experimental changes in tonicity are produced by intra-arterial infusions of hypertonic or hypotonic solutions changes in the vascular resistance will occur (Lundvall, 1972). The cellular events behind vascular responses to changes in osmolality have been elucidated by Jonsson (1970) who investigated the effects of alterations in extracellular osmolality on the electrical and mechanical activity of the rat portal vein. In these studies it was found that media made hypertonic by adding sucrose caused shrinkage of the smooth muscle cells and a reduced spontaneous electrical and mechanical activity. Hypotonic solutions caused swelling of the cells and increased spontaneous activity both electrical and mechanical (Johansson and Jonsson, 1968). The muscle cells were found in general to behave like perfect osmometers (Arvill, Johansson and Jonsson, 1969) and the changes in activity were thought to be caused by variations in transmembrane ionic concentration gradients or changes in membrane permeability (Jonsson, 1970).

Stretch of muscle cells in the walls of resistance and precapillary sphincter vessels caused by an increase in transmural pressure gives enhancement of contractile activity. This kind of response is the background of the so-called myogenic theory of blood flow autoregulation (for ref see Folkow, 1964; Mellander and Johansson, 1968; Johnson, 1974). Recent works on the vascular bed of skeletal muscle have shown that the vascular response is particularly intense during the dynamic phase of changing pressure (Mellander

et al., 1980), suggesting a rate sensitive element in the myogenic reaction.

The rat portal vein has also been used as an in vitro model for the study of the mechanisms behind this myogenic response. In a study by Johansson and Mellander (1975) graded dynamic responses to different rates of length changes were found. There was an increased electrical and mechanical activity during stretch. The contractile activity was measured in this study as contractile force (isometric recording), but in view of the fact that myogenic reactions in vivo must be associated with changes in vessel caliber it was also of interest to study the corresponding effects when the muscles were allowed to undergo active shortening (isotonic recording) during stretch.

3.2 Outline of methods

The responses to stretch and changes in osmolality were studied by the sucrose-gap technique. The mechanical activity was recorded directly and quantified by integrating the active tension curve. The electrical spikes were recorded in DC and/or AC mode (Johansson and Mellander, 1975) and the number of spikes were counted electronically.

The osmolality of the test solutions was varied by adding sucrose. K^+ -high solution used to depolarize the muscle was made by replacing all NaCl with an equimolar amount of KCl.

The change of length of the muscles was produced in two different ways. In the first type the muscle was mounted between a fixed muscle holder and a force transducer which could be raised and lowered at graded but constant rates. Under these conditions the active contractions of the preparation appeared as increases in force. In the second type of experiment the preparation was connected to an isotonic lever supplied with a photoelectric device which permitted recording of passive and active changes of muscle length. The load on the lever i.e. the passive force applied to the muscle, could be varied gradually via a spiral spring connected to the lever axis. Active contraction in this case manifested itself as shortening against the afterload represented by the force of the spring. It was possible to shift directly from one recording system to the other without change in initial length or preload of the muscle.

3.3 Results and comments

3.3.1 Osmolality (Paper IV)

In normal solution the portal vein has regular spontaneous activity composed of mechanical contractions of the frequency of 2-3 min associated with bursts of spikes. If the osmolality of the solution is increased by adding sucrose, which appears not to enter the cell in any significant amount (Arvill, Johansson and Jonsson, 1969), there is a decrease in both electrical and mechanical activity during the first 10 min period. After 10 min the activity increased again and reached a stable level after 30 min. The effects of increasing the osmolality to 390 mosm/kg are summarized in fig 8A. Both the integrated mechanical activity and the number

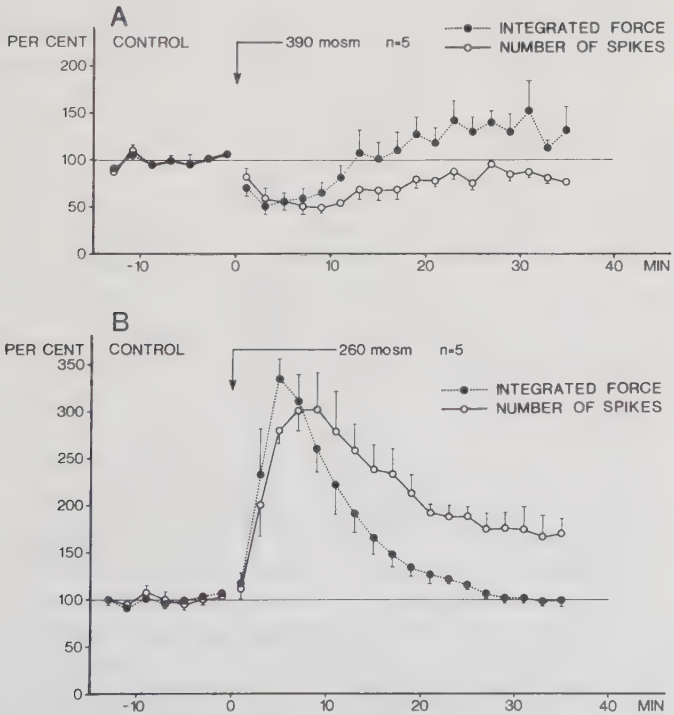


Fig. 8 Change in integrated mechanical activity and number of spikes in response to hyperosmolality, 390 mosm/kg (A) and hypoosmolality, 260 mosm/kg (B). The data are expressed in per cent of the values obtained in control medium (290 mosm/kg). Mean values $\pm$ S.D.

of spikes decreased by about 50% during the first part but after 30 min the number of spikes was about 90% and the integrated mechanical force about 135% of control.

If the osmolality was lowered there was an increased frequency of bursts and the contraction frequency was correspondingly enhanced during the first 10 min. These effects then diminished with time. The results from 5 experiments of lowering osmolality to 260 mosm/kg are shown in Fig. 8B. The integrated force is increased to about 330% and the number of spikes to about 300% during the first 10 min period. The activity then decreases and after 30 min the integrated force is at control level but the number of spikes is still 170% above control. To get some idea of the mechanical effectiveness of each spike, the ratio of integrated force per spike was calculated. Hyperosmolality decreased this ratio during the first 10 min but later increased it to about 160% of control. Hypoosmolality increased this ratio during the first 10 min period but then reduced it and after 30 min it was about 60% of control.

The effect of osmolality on the amplitude of K^+ -contractures was examined. Such contractures are independent of spike discharge and it was of interest therefore to see if this mechanical response would also be affected by changes in tonicity. When the muscle was exposed to hyperosmolality (390 mosm) the first contracture elicited after 15 min was unchanged but later the contractures increased in amplitude to about 125% of control. On return to normal osmolality the first contracture was high (132%) but the force then decreased to the same level as the initial control contractures. In hypoosmolality there was first a tendency for the amplitude to increase but later to decrease to about 80% of control. On return to normal osmolality the amplitude was restored. By further analysis of the data on K^+ -contractures it appeared that relaxation was much faster in hyperosmolality. The time required to reach 50% force was only about 40% of the time in control situation. In hypoosmolality this relaxation time was increased to about 151% of control. These data indicate that the sequestration and/or extrusion of Ca^{2+} is more effective in hyperosmolar than in isoosmolar solution and less effective in hypoosmolality.

3.3.2 Stretch

In these experiments the electrical and mechanical responses of the rat portal vein to passive stretch and shortening were examined. The muscles were stretched in two different ways. In the first type of experiment the muscle was subjected to changes in length at a constant dL/dt i.e. constant length change per unit time. The muscle developed contractile force without active change in length. It was stretched at different rates (mm/min) and then passively shortened at the same rates.

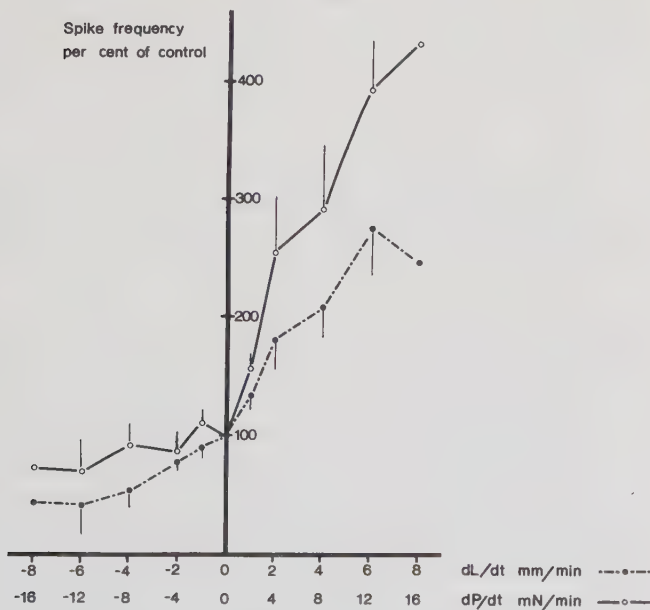


Fig. 9 Electrical response (mean $\pm$ S.E.) of the isolated rat portal vein to dynamic changes in length or passive force at rates ranging from -8 to +8 mm/min or -16 to +16 mN/min, respectively. Spike activity at control length was set to 100%. The values for dL/dt are the absolute rates of length change applied in the experiments: the figures for dP/dt are given as mean values since passive compliance varied between the different muscles.

In the second type of experiment the muscle was stretched to the same final length and passive force as the first type, but in this case the stretch was applied by increasing the load on a lever at a constant rate i.e. at constant dP/dt . Here the muscle shortened actively during the phasic contractions. In both procedures the total increase in length was about 40% of the muscle's starting length. The initial preload was about 0.7 mN and increased to about 4 mN.

Fig. 9 shows the change in electrical activity obtained during the dynamic phases of stretch and shortening in the two types of experimental procedure. The change in activity is given in per cent of the mean frequency of spikes in the control period preceding each stretching of the muscle. The stretching of the muscle, whether it was made by increase in length at a constant rate (dL/dt) or by increase in force at a constant rate (dP/dt) caused an increase in spike activity. The response was graded so that the faster the change the more intensive the activity. Shortening of the muscle (negative dL/dt or dP/dt) inhibited the spike activity. The results show that even though active shortening could take place during the second type of experiment (dP/dt) spike activity was markedly increased by the dynamic stretch.

Further analysis of the data was carried out to try to differentiate between the role of dL/dt and dP/dt . By comparing the spike activity during the first and the second half of the dynamic period it was found that during increase in force at a constant dP/dt , the spike activity was similar in both halves even though dL/dt was steadily decreasing. During increase in length at a constant dL/dt , the spike activity in the second half increased to 166% of that in the first half. During this stretch period dP/dt steadily increased. These findings indicate that stimulation of spike activity during stretch is more closely related to the change in passive force, dP/dt , than to applied increase in length, dL/dt .

The electrical activity during the period at constant increased length or force (static stretch) was only slightly enhanced, or about 10%, compared to control.

3.4 Discussion

There are three main factors related to tissue metabolism that seem to deserve particular attention as mediators of vasodilation in exercise hyperemia (Mellander and Johansson 1968). These are lack of oxygen, which will be discussed in the next section, change in extracellular potassium, and increase in tissue osmolality. It has been shown that exercise even at moderate levels, can cause hyperosmolality which simultaneously induces a decrease of vascular resistance (Mellander et al., 1967). In the same study it was shown that hyperosmotic infusion caused vasodilation to a similar extent as increase in osmolality by exercise. Similar results are reported in a later study by Lundvall (1972). He also found that the dilator response of the resistance vessels to hypertonicity was followed by a secondary partial recovery. He concluded that the observed vascular effects of hyperosmolar environments were caused by direct inhibition of the vascular smooth muscle tone.

Studies of isolated rat portal vein (for ref see Jonsson 1970) indicated that hypotonic media caused swelling and excitation of the spontaneous activity, hypertonic solution caused shrinkage of the cells and inhibition of activity. However, most of these in vitro experiments were concerned with rather short term (5-15 min) effects of the anisomolar solutions. In a more recent study (McKinley et al., 1974) where the preparation was exposed to anisomolar solution over longer periods, a secondary effect of enhanced amplitude of the mechanical contractions was found in hyperosmolality and reduced contractile force in hypoosmolality. By integrating the active force over time in our experiments we take into account both changes in amplitude, duration, frequency and form of the individual contractions. The changes in electrical activity were also studied. Our result supports in some respects the findings of Jonsson (1970) and McKinley (1974). There is a decrease in number of spikes and integrated mechanical activity during the first 10-15 min period in moderate hyperosmolality, but the spike discharge then returns to control level whereas the mechanical activity increases above control. This means that the mechanical efficiency of the spikes increases during prolonged exposure to hyperosmolality. During hypoosmolality the electrical and mechanical activity increases during the first 10-15 min but decreases

thereafter. The integrated mechanical activity returns to control whereas the electrical discharge remains elevated implying that the efficiency of the spikes is decreased in hypoosmolality.

Mellander et al. (1967) found an inhibitory action of hyperosmolality on vascular smooth muscle in vivo and in vitro. They showed that the inhibition of spike activity in the isolated preparations could be counteracted by increase in extracellular K^+ , but suggested simultaneously that other factors than changes in the K^+ gradient were involved. Jonsson (1970) investigated further the effects of osmolality on ionic fluxes in isolated vascular smooth muscle. He concluded that the inhibition of the portal vein in hypertonicity with non-permeant molecules like sucrose and the excitation in hypoosmolality are due to changes in ionic gradients over the membrane and in ionic permeability associated with cell shrinkage and swelling. These effects on spike discharge may be most pronounced directly after change in osmolality and may be compensated for during prolonged exposure even if changes of cell volume persist.

Our present results show that anisomolar solutions also change the relation between electrical and mechanical activity in such a way that the active force per spike increases with prolonged exposure to hyperosmolality and decreases in hypoosmolality. These "inotropic" effects are further reflected in the changes in contracture amplitude in the depolarized preparations. The mechanisms behind these changes in the relation between electrical and mechanical events are not at all clear. As shown by Andersson et al., (1974) a strongly hypertonic media produced a tonic contraction in the portal vein. This contracture is only moderately dependent on $[Ca^{2+}]_o$ but is abolished in $Ca^{2+} - Mg^{2+}$ free solution (Andersson et al., 1974; Hellstrand and Arner, 1980). It is possible that the influence produced by prolonged exposure to the moderate changes in osmolality on the mechanical effectiveness of the spike and force produced by depolarizing solution in our experiments, may be mediated by the unknown cellular changes which lead to the slow contracture at marked hyperosmolality.

It has been suggested earlier that the electrical responses of the portal vein to swelling and shrinkage in anisomolar solutions and the responses to passive stretch and shortening may reflect a common mechanism of mechano-electrical coupling in the cell membrane of this vascular smooth muscle (Johansson, 1971). Therefore these two kinds of responses to cell deformation have been treated together in this section.

The suggestion that changes in transmural pressure may cause variations in vascular tone was put forward by Bayliss (1902). This idea has received strong experimental support in more recent years (Folkow, 1962; Johnson, 1964; Mellander and Johansson, 1968; Grände, 1979) and the myogenic mechanism is now widely accepted as an important factor in control of vascular tone. Recent *in vivo* work indicates that the vascular response to a rise in transmural pressure is much more vigorous during the dynamic phase of changing pressure than during sustained elevated pressure (Grände et al., 1977). These recent results indicate that a rate sensitive element is involved in the myogenic reaction.

In an *in vitro* study on isolated vascular smooth muscle the cellular mechanism behind such dynamic reactions was elucidated (Johansson and Mellander, 1975). The results showed pronounced and graded dynamic responses to different rates of length changes in the rat portal vein. Stretch caused excitatory effects, and inhibitory effects were observed during passive shortening of the preparation. A static increase in length caused only a minor increase in activity. In view of the fact that myogenic reactions *in vivo* must lead to changes in vessel caliber in order to mediate for instance autoregulation of blood flow, a series of experiments was performed on the portal vein where the muscle could counteract the applied stretch by active contractile shortening. The results show that the electrophysiological response to stretch was similar when the muscle could shorten actively as when the contraction manifested itself purely as force. If the rate of change in length was increased the responses (isometric or isotonic) changed in a similar manner (Fig. 9). It was therefore suggested that active shortening neither abolishes nor reduces the excitatory action of the passive dynamic stretch. The results from the present study seem therefore compatible with the *in vivo* findings of myogenic autoregulation with

regard to both dynamic and static stimuli (Basar and Weiss, 1969; Gründe et al., 1977).

The further analysis of the responses to increase in length at a constant rate (dL/dt) or increase in passive force at constant rate (dP/dt), indicated that the excitatory response to dynamic stretch was more closely related to dP/dt than dL/dt . Work on sensory mechano-receptors supports this suggestion as they have indicated that the receptors are primarily stimulated by change in tension rather than by change in length (Teorell, 1971). Further experiments are needed to define the nature and localization of the sensing element and also to find out whether identical or related membrane mechanisms are involved both in the response to stretch and in the response to osmotic swelling.

4. HYPOXIA

4.1 Introduction

Oxygen tension is one of a number of different factors related to tissue metabolism which have been suggested as mediators of functional hyperemia in skeletal muscle (Mellander and Johansson, 1968). Variation in P_{O_2} in the vessel's environment has been proposed to regulate blood flow through a direct influence on vascular tone (Carrier et al., 1964). If the local control of blood flow is to be explained by a direct effect of oxygen tension, then the contractile activity of the vessels must be affected by variations in P_{O_2} . Hellstrand, Johansson and Norberg (1977) studied these effects on the myogenic activity in the rat portal vein. They found that the spontaneous activity was well sustained when oxygen tension was lowered from 670 mm Hg (96%) to about 50 mm Hg. If P_{O_2} was further decreased there was a graded linear inhibition of integrated mechanical activity. At $P_{O_2} < 5$ mm Hg spontaneous activity usually disappeared. There was a similar decrease in spontaneous electrical activity during exposure to hypoxia. However, about 25% of the response to K-high solution (120 mM) remained even in extreme hypoxia. It was suggested therefore that the effect of hypoxia in the polarized muscle was a combined result of reduced spontaneous impulse generation and depressed contractile activity.

The purpose of the studies considered in this section was to elucidate how vascoactive drugs and ionic changes affect the hypoxic response. Attempts have also been made to clarify the mechanisms by which membrane function is affected by low P_{O_2} and these attempts encompass measurements of transmembrane K^+ fluxes and recording of absolute membrane potentials by intracellular microelectrodes.

4.2 Outline of methods

The effect of hypoxia on the electrical and mechanical activity was examined mainly in two different ways, by the sucrose gap technique (Paper VI) and by using intracellular microelectrodes (Paper VII). The extracellular sucrose gap method in a well controlled experiment can be used to record the electrical signals as compound action potentials,

which makes it possible to study the relationship between electrical and mechanical activity. Under particular circumstances it may even be possible to obtain absolute values of membrane potentials from sucrose gap recordings (Bennet and Burnstock, 1966). However, intracellular microelectrodes are for the present time the only technique available for obtaining reliable values of the membrane potential and potential changes. This method can also be used to study the relationship between electrical and mechanical activity but only if the preparation behaves as a uniform single unit i.e. if the membrane response of the impaled cell is representative of the population of the force generating cells.

Krebs solution was used in both types of experiment. In the sucrose gap experiments the "normoxic" solution was bubbled with a gas mixture of 96% O_2 and 4% CO_2 giving a P_{O_2} of about 670 mm Hg. In the microelectrode experiments the Krebs solution was bubbled with a gas mixture of 16% O_2 , 80% N_2 , and 4% CO_2 giving a control P_{O_2} of about 115 mm Hg, approximately corresponding to that of alveolar air. According to Hellstrand et al. (1977) very small changes were observed in the spontaneous activity of the portal vein when P_{O_2} was lowered from 670 to 115 mm Hg, indicating sufficient oxygenation in both solutions. Hypoxia was produced in all cases by bubbling the solution with 96% N_2 and 4% CO_2 . This reduced the P_{O_2} in the perfusion solution to 5 mm Hg in the sucrose gap experiments but only to about 10 mm Hg in the microelectrode experiments because of oxygen leakage from the environment. Solution with increased K^+ was prepared by substituting 18 mM KCl for equimolar amounts of NaCl. In solution with reduced Na^+ , 61 mM NaCl were replaced by equiosmolar amounts of sucrose.

4.3 Results and comments

In the sucrose-gap and microelectrode experiments the spontaneous activity of the rat portal vein was composed of regular contractions of a frequency of 2-3 per min associated with bursts of spikes. This kind of activity can be maintained for hours in both types of experiments and is comparable to that normally encountered in ordinary muscle baths.

4.3.1 Sucrose-gap experiments

If the perfusion solution was switched from a normoxic Krebs solution to a hypoxic one, which had been bubbled with 96% N_2 and 4% CO_2 , the electrical and mechanical activity decreased and disappeared within 5-10 min (Fig. 10). The rate of the decrease was dependent on the flow rate of the test solution. During the decline the contraction frequency was not markedly affected but on the average it was observed that the integrated force decreased faster than the number of spikes (Fig. 10). On return to normoxic solution the spontaneous activity was restored in a few minutes, but in some experiments a short period of increased activity could be seen immediately after return to normoxic conditions.

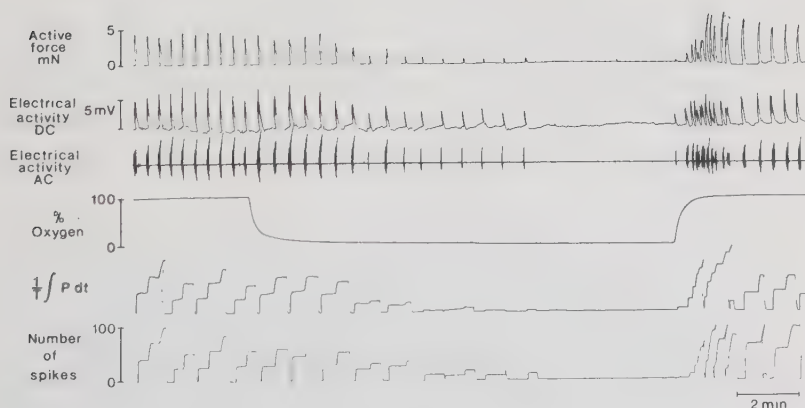


Fig. 10 Effect of hypoxia ($PO_2 < 5$ mm Hg) on the spontaneous electrical (DC and AC) and mechanical activity in the rat portal vein. Mechanical and electrical activity was quantified as integrated active force and number of spikes per min.

During the exposure to hypoxia when all activity had disappeared the muscle cells were very sensitive to changes in P_{O_2} . If a minor amount of oxygenated solution was added to the perfusion solution, the preparation responded immediately with reappearance of mechanical and electrical activity. It was also possible to reinitiate the spontaneous activity during maintained hypoxia by adding stimulating agents such as noradrenaline (10^{-6} M), acetylcholine (10^{-5} M), 4-aminopyridine (10^{-3} M), and Ba^{2+} (0.3 mM). Adding 10^{-6} M noradrenaline caused phasic contractions associated with bursts of spikes. Under normoxic conditions this same concentration of noradrenaline caused continuous spiking and tetanic contractions. It seems therefore that higher concentrations of the stimulator are needed during hypoxia (see also Hellstrand et al., 1977).

A shift to a Krebs solution with moderately increased $[K^+]$ (24 mM) or to a solution with decreased $[Na^+]$ (76.5 mM) also reinitiated the spontaneous activity during hypoxia. Under normoxic conditions the integrated mechanical activity was measured to 260% of control in K^+ -high solution and to 165% in Na^+ -low solution (Fig. 11). If these solutions were made hypoxic the integrated mechanical activity was decreased but not to the same extent as in normal Krebs. During prolonged exposure the activity recovered and reached 50% of control in K^+ -high solution and 250% in Na^+ -low solution after 2 h. A small recovery was also seen during 2 h exposure in hypoxic normal Krebs but only to about 10% of control.

4.3.2 $^{42}K^+$ experiments

To examine the effects of hypoxia on the membrane permeability characteristics for potassium, uptake and efflux experiments were done. In uptake experiments, the preparations were incubated for 15, 30, 60, and 120 min in $^{42}K^+$ containing hypoxic or normoxic Krebs solution. The initial uptake (after 15 and 30 min) was similar in hypoxic and normoxic solutions but after the two longer periods (60 and 120 min) the amount of tissue potassium equilibrated with the tracer was somewhat, but not significantly, smaller in hypoxia. The amount of $^{42}K^+$ after 2 h corresponded to about 45 mM K^+ /kg tissue, which is similar to that obtained by Haljamäe et al. (1970) and Wahlström (1971).

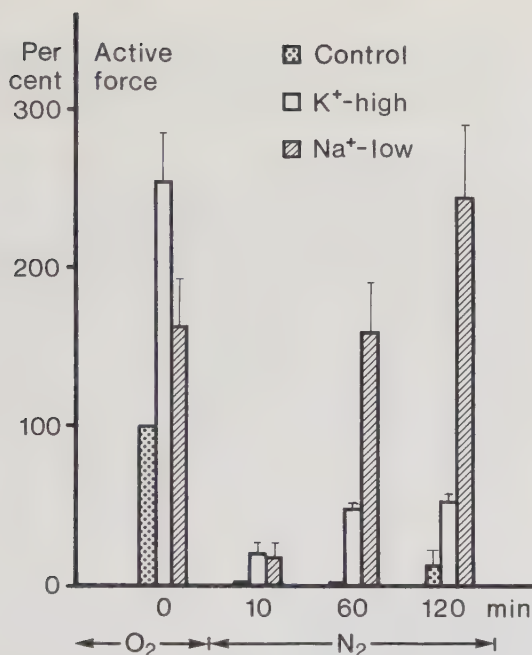


Fig. 11 Integrated active force in normal, K⁺-high (24 mM), and Na⁺-low (76.5 mM) solutions (means $\pm$ S.D.) and the effect of prolonged hypoxia (2 h). The activity in normoxic, normal solution is set to 100%.

Washout of $^{42}\text{K}^+$ from preparations equilibrating for 2 h in $^{42}\text{K}^+$ containing Krebs solution was studied. The fractional release of the isotope during the 2 min sampling intervals was measured. The results obtained showed clearly that exposure to hypoxia decreased the efflux of potassium, and a return back to oxygenated solution increased it again. In order to compare this inhibitory effect of hypoxia with some other condition where the spike activity was abolished, Mn^{2+} ions (0.4 mM) (Collins et al., 1972b) were added to the perfusion solution. The spontaneous activity was inhibited and a similar decrease in the efflux of $^{42}\text{K}^+$ was seen as in hypoxia. No additional effects on efflux appeared in response to hypoxic Mn^{2+} containing solution. A return to

oxygenated Mn^{2+} -free medium restored the spontaneous activity and the fractional release of $^{42}K^+$.

4.3.3 Microelectrode experiments

The spike bursts recorded from the single cells could be subdivided into two categories. One which might be regarded as a pacemaker type with prepotentials, where spike activity appeared before the onset of the contraction. The other might be regarded as a follower type, lacking distinct prepotentials and spike activity appearing at or after the onset of the contraction. The spikes within each burst varied somewhat in amplitude and duration; some had overshoot others had not. The

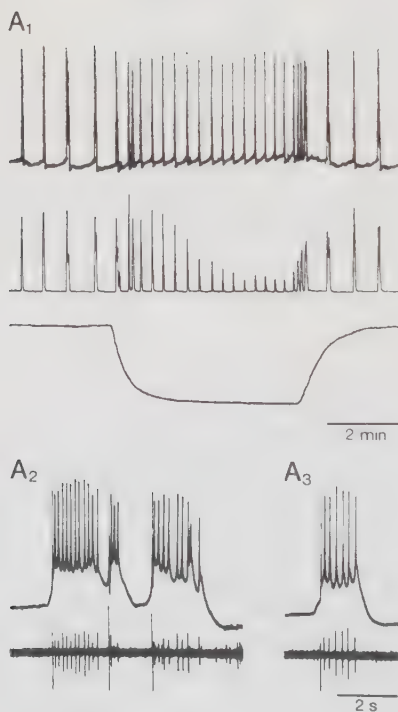


Fig. 12 A₁, pen recordings of electrical and mechanical activity in varying oxygen tension. A₂ and A₃ oscilloscope recordings of individual spike bursts from A₁, and their time derivative obtained in normoxic (A₂) and hypoxic (A₃) conditions.

mean potential between the bursts of action potential, the "resting" potential, was -58.0 ± 0.1 (mean $\pm$ S.E., $n=30$).

The effect of short exposure to hypoxic solution is shown in Fig. 12. There was a marked decrease in amplitude and duration of the phasic contractions, and the bursts of spikes were shortened but spike amplitude was unchanged. An increase in the frequency of the spontaneous activity was often seen. The "resting" membrane potential was -60.0 ± 1.5 mV (mean $\pm$ S.E., $n=9$) which is not significantly different from the results in normoxic conditions. During prolonged hypoxia the mechanical activity decreased further, the spikebursts became shorter and there was a tendency to desynchronization of the electrical and mechanical activity. The efficiency of the spikes became less as shown by the ratio: force per spike which was only about 31% of control (in normoxic condition).

An increase in $[K^+]_o$ to 24 mM during hypoxia depolarized the membrane (about 10 mV) and increased the burst frequency but each burst was composed of fewer spikes. Despite the decreased duration of spike discharges the amplitude of the phasic contraction was strongly augmented, and the ratio force per spike was increased to 164% of its control value in normoxic condition.

A decrease in $[Na^+]_o$ to 76.5 mM hyperpolarized the membrane (by about 10 mV), but the bursts appeared more frequently, were shorter and contained fewer spikes which also had slower time course. There was also a tendency to desynchronization.

Lowering of $[Ca^{2+}]_o$ from 2.5 to 1.0 mM in normoxic solution did not affect the resting membrane potential (-58.0 ± 0.6 mV) (mean $\pm$ S.E., $n=10$), but there was shortening of the spike bursts and increase in their frequency. The spikes in each burst had lower amplitude and slower time course. However, the most conspicuous effect of the lowered $[Ca^{2+}]$ was reduction in amplitude and duration of the phasic contractions. The responses to hypoxia and to increased $[K^+]_o$ and lowered $[Na^+]_o$ during hypoxia in low $[Ca^{2+}]$ were qualitatively similar to those

seen in normal $[Ca^{2+}]_o$. However the mechanical activity in hypoxia disappeared faster, and in prolonged hypoxia there was a tendency to abolishment of the spike activity.

4.4 Discussion

It has been known for some time that low oxygen tension can cause relaxation in vascular smooth muscle. Guyton et al. (1964) suggested that oxygen lack in the tissue affected the vascular smooth muscle cells directly and caused relaxation. This idea was further supported by Fairchild et al. (1966) who showed that there was no recovery from reactive hyperemia if the vessels were perfused with oxygen depleted blood. The results obtained from experiments on isolated vascular smooth muscle indicated that P_{O_2} in the physiological range (100 - 30 mm Hg) affected the vascular resistance directly and that hypoxia caused inhibition of the noradrenaline induced vascular tone (Carrier et al. 1964). Later Detar and Bohr (1968) concluded from their experiments on rabbit aorta that the observed immediate dependence of the contractile tension on P_{O_2} could be explained by the metabolic role of O_2 . P_{O_2} in the physiological range could serve as a control for the formation of high-energy intermediates necessary for cellular processes. More recent studies have shown that exposure to hypoxia decreased the ATP and phosphocreatine content in the rabbit aorta (Namm and Zucker 1973) and the rat portal vein (Hellstrand et al. 1977). It is therefore possible that the hypoxic inhibition of activity depends on decreased access to substrate (Mg-ATP) for the contractile machinery.

Recently it was pointed out that the O_2 consumption of the smooth muscle will cause P_{O_2} gradients within the vessel wall and in unstirred layers around the preparation causing hypoxic conditions in its central core even at relatively high surrounding P_{O_2} (Pittman and Duling, 1973, Fay et al. 1977). However, other studies on portal vein indicated that the drop in P_{O_2} from the stirred bathing solution to the core of the preparation was only about 20-25 mm Hg while there was a 50 mm Hg range for the effects of surrounding P_{O_2} on muscle activity (Hellstrand et al. 1977, Hellstrand, 1978). Hellstrand et al. (1977) found that during low P_{O_2} when the spontaneous activity was completely inhibited it was still possible to elicit K^+ -contractures with

25% of normal amplitude. This indicates that there are still energy supplies available even when the myogenic activity is suppressed by low P_{O_2} .

Electrical recordings during spontaneous activity indicate that the electrical activity has high sensitivity to changes in P_{O_2} and that these effects are then reflected in the mechanical activity. The aim of the studies presented in this section was to elucidate further the sensitivity to variation in P_{O_2} .

Our observations of the inhibitory action of hypoxia on the electrical and mechanical activity of the rat portal vein confirm the findings of Hellstrand et al. (1977). However, during hypoxia, when all the activity had disappeared, it was still possible to reinitiate the spontaneous activity by adding various stimulating agents such as noradrenaline, acetylcholine, 4-aminopyridine and Ba^{2+} . The responses to these agents were, however, markedly inhibited compared to those obtained under normoxic conditions. One of the excitatory effects of noradrenaline is considered to be an increase of membrane conductance mediated via permeability to several ions (Wahlström 1973). Other agents like 4-aminopyridine and Ba^{2+} are suggested to reduce conductance by blocking K^+ channels (Hermsmeyer, 1976, Yeh et al. 1976). For these and other reasons it was of interest to investigate if specific permeability changes to ions lie behind the hypoxic inhibition. Our results showed that the uptake of $^{42}K^+$ was not different in normoxic and hypoxic media but the efflux was decreased under hypoxic conditions. However, this reduced efflux was most likely linked to the abolition of spikes during hypoxia as similar effects were obtained by addition of Mn^{2+} (for ref see Weiss 1977). These results therefore speak against any specific action of hypoxia on K^+ permeability.

To get more knowledge about the role of the membrane in the hypoxic inhibition direct measurements of the resting membrane potential and spike activity were obtained by microelectrodes. The sucrose gap experiments had indicated a small depolarization during hypoxia but this extracellular technique does not permit any definite conclusion in this respect (Hellstrand et al. 1977). In the microelectrode experiments a hypoxia

severe enough to inhibit the spontaneous activity completely was not obtained but the contractile activity was inhibited to 90%. No significant change in the resting membrane potential was observed during hypoxia. This result does not, however, rule out the existence of an increased depolarizing drive which may be obscured by the relatively strong after-discharge hyperpolarization seen in hypoxia.

The observation of maintained normal degree of membrane polarization in hypoxic condition is supported by recent studies, which have shown that the activity of the Na-K pump in vascular smooth muscle can be sustained by energy supplied by anaerobic glycolysis (Casteels and Wuytack, 1975, Paul et al. 1979).

Even though the membrane potential was not affected by hypoxia the sensitivity of the membrane activity to change in P_{O_2} seems large. Lowering of P_{O_2} decreased immediately the duration of the bursts and the number of spikes per burst before any effects were observed on the mechanical contractions (Sigurdsson and Grampp, unpublished observation). However, the mechanical activity was then inhibited faster than the electrical discharges during further increase in hypoxia, and was even abolished while considerable membrane activity was still observed. In the micro-electrode experiments with the lowest P_{O_2} and in hypoxic low Ca^{2+} -solution a clear tendency to abolition of the electrical activity was observed.

Hypoxia therefore seems to exert some of its inhibitory effects through those membrane mechanisms which are responsible for pacemaker activity and spike generation. An electro-mechanical uncoupling acting simultaneously with the membrane mechanism is also an important factor as indicated by the decrease in active force per spike during hypoxia. The Ca^{2+} ion is the suggested mediator in the electro-mechanical coupling and it is possible that this function becomes insufficient in hypoxia. This could be caused in the following three ways. Firstly by insufficient influx of Ca^{2+} in connexion with the discharge activity, which seems unlikely as the configuration of the spikes was not changed. Secondly by insufficient release of Ca^{2+} from the intracellular stores

and thirdly by changed affinity of the regulatory system for Ca^{2+} . Both the second and third situation could occur in hypoxia because of intracellular acidification and/or accumulation of metabolites. Such a pH decrease could be expected in the portal vein exposed to hypoxia because the lactate production is increased 2.7 times that in normoxia (Hellstrand 1977b). Increase in extracellular PCO_2 which should cause a reduced intracellular pH, decreases smooth muscle activity (Grün and Fleckenstein, 1972, Paul and Rüegg, 1978, Linke et al. 1979). Direct support for an influence of intracellular acidification is provided by the demonstrated shift of the Ca^{2+} dependence of the actomyosin ATP-ase to higher Ca^{2+} -values at low pH (Mrwa et al. 1974). To verify the role of pH in the hypoxic effects, measurements of intracellular pH during the actual conditions are needed.

Increased $[\text{K}^+]_o$ during hypoxia resulted in depolarization and increased activation of the membrane besides activation of the contractile system leading to marked increase in contractile force. This increase in force could be caused by increased intracellular Ca^{2+} . Droogmans et al. (1977) have shown that an increase in $[\text{K}^+]_o$ facilitates the penetration of Ca^{2+} into intracellular compartments. An increase in $[\text{Ca}^{2+}]_i$ could also explain the effect of lowered $[\text{Na}^+]_o$. It could be caused by increased Ca^{2+} influx due to diminished competition between Na^+ and Ca^{2+} for the same membrane channels (Droogmans et al. 1977) or by a reduced Ca^{2+} efflux via the Na-Ca exchange mechanism (Reuter et al. 1973, Blaustein, 1977). The activated electrical activity during the hyperpolarized state in Na^+ -low solution is difficult to explain and needs further experiments for clarification. The increase in mechanical activity during prolonged hypoxia (Fig.11) in normal, K^+ -high or Na^+ -low solutions may be an adaptation to the hypoxic conditions of the kind earlier studied and discussed by Detar and Bohr (1972).

6. GENERAL DISCUSSION AND SUMMARY

A major part of this discussion will deal with the main conclusions drawn in each of the papers. More specific discussion points have been treated in the discussions belonging to the respective chapters.

In a single unit smooth muscle the action potential is almost undoubtedly triggering the contraction, and during the action potential the Ca^{2+} -ion seems to be the main component of the inward current. As link between the action potential and the contractile processes the Ca^{2+} -ion is the most probable candidate. However, the influx of Ca^{2+} during the action potential seems to be insufficient to activate the contractile mechanism. It is therefore suggested that these Ca^{2+} -ions are able to release Ca^{2+} from superficial or intracellular bound pools. In that way it is possible to increase $[\text{Ca}^{2+}]_i$ well above the threshold for activation, which is suggested to be about 10^{-7} M. Maximum tension is reached at $[\text{Ca}^{2+}]_i$ around 10^{-5} M. Relaxation is brought about by sequestration of the Ca^{2+} -ions into cell organelles such as sarcoplasmic reticulum and mitochondria and/or rebinding to the cell membrane. Some extrusion of Ca^{2+} to match the influx must also take place.

It has been suggested that contraction and relaxation of smooth muscle may occur without changes in resting membrane potential or action potential frequency. This so called pharmacomechanical coupling could act simultaneously with the electromechanical processes discussed above (for ref see Somlyo and Somlyo, 1968; Johansson and Somlyo, 1980).

The divalent ions Sr^{2+} and Ba^{2+} seem to be able to substitute for Ca^{2+} in the inward current, to release bound Ca^{2+} from the pools and even to act directly by themselves at the regulatory sites; this is especially valid for Sr^{2+} . The sequestration and extrusion mechanisms seem to be unspecific enough to handle Sr^{2+} and Ba^{2+} as well as Ca^{2+} . However the divalent Mg^{2+} -ion is not able to replace Ca^{2+} in any of these mechanisms. Added Mg^{2+} seems to depress the excitability of the cell membrane and even to disturb Ca^{2+} -permeability and cause inhibition of the spontaneous activity. Mg^{2+} depletion causes also inhibition of the contractile

activity possibly through lack of substrate (Mg-ATP). the contractile activity is initiated by spontaneous action potentials from pacemaker cells in the single unit smooth muscle and the action potentials are then spread electrotonically from cell to cell. the frequency of the action potentials and the "efficiency" of the ion mechanisms which they activate can be affected by various local factors. Changes in the ionic environment (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) can affect the membrane properties and the ionic mechanisms directly. Changes in osmolality of the extracellular fluid affects the volume of the cell. Increased volume stimulates the spontaneous activity and decreased volume inhibits it. These stimulatory and inhibitory responses can be mediated by changes in the ion concentrations and/or permeability changes in the membrane. Some adaptation occurs during prolonged exposure to changed osmolality as the effects fade away with time. There is an increased "effectiveness" of the electrical spikes during prolonged hyperosmolality and decreased during hypoosmolality. Changes in membrane conductance mediating the electrophysiological responses to osmotic cell deformation may also be involved in the changes of membrane activity elicited by passive stretch or shortening. Rate sensitivity characterizes both the osmotic responses and the responses to changes in length. Active contractile shortening does not interfere with the excitatory influence of stretch which appears to relate to passive dP/dt .

Hypoxia seems to regulate vascular tone by affecting both electrical and mechanical activity. During severe hypoxia a complete inhibition of the mechanical and electrical activity is observed. This inhibitory effect can be counteracted by some stimulating agents and by ionic changes such as increased $[\text{K}^+]_o$ and decreased $[\text{Na}^+]_o$. No change in resting membrane potential is observed during the hypoxia. It is suggested that the inhibitory responses to hypoxia are combined effects of shortage of substrate for different cellular processes, of depressed membrane excitability and an ineffectiveness of some link in electro-mechanical coupling, perhaps caused by change in intracellular pH.

SUMMARY

The present studies on the isolated portal vein from rats have led to the following conclusions:

1. Extracellular Ca^{2+} is required to trigger the release of superficially bound Ca^{2+} causing the fast phase in the response to K^{+} -depolarization. The extracellular Ca^{2+} is depleted in about 3 min; the superficial pools are emptied during the first 30 min in Ca^{2+} -free solution. The slow phase of the contracture obtained after depletion of the superficial pools uses mainly extracellular Ca^{2+} .
2. Sr^{2+} and Ba^{2+} are able to substitute for Ca^{2+} in spontaneous electrical activity. Excitation-contraction coupling is less effective when Ca^{2+} is replaced by Sr^{2+} or Ba^{2+} . The mechanical responses in Sr^{2+} and Ba^{2+} solutions appear to be combined effects of liberation of tissue bound Ca^{2+} and a direct action.
3. Mg^{2+} cannot substitute for Ca^{2+} in the spontaneous electrical and mechanical activity. Instead, Mg^{2+} depresses the electrical excitability of the cell membrane and even interferes with intercellular propagation.
4. A rate dependent response to stretch and shortening was observed in isolated vascular smooth muscle. This response was similar under isometric and isotonic conditions. The excitatory response to dynamic stretch is more closely related to dP/dt than to dL/dt .
5. Hyperosmolality inhibited and hypoosmolality excited the spontaneous electrical and mechanical activity. These responses faded during prolonged exposure. The "mechanical effectiveness" of the spikes decreased during prolonged exposure to hypoosmolality and increased in hyperosmolality.
6. The electrical and mechanical activity was abolished in severe hypoxia ($P_{O_2} < 5$ mm Hg). The activity could be reinitiated during hypoxia by various stimulating agents and by increase in $[\text{K}^{+}]_o$ and decrease in $[\text{Na}^{+}]_o$. The uptake and efflux of $^{42}\text{K}^{+}$ seemed not to be affected directly by hypoxia.
7. Hypoxia did not change the membrane potential significantly. Moderate hypoxia ($P_{O_2} \leq 10$ mm Hg) inhibited the mechanical activity more than the electrical activity. It is suggested that hypoxia exerts its inhibitory action through a combined effect of depressed electrical activity and an electro-mechanical uncoupling which is possibly a pH dependent effect.

ACKNOWLEDGEMENT

I would like to thank all the people in the department who have supported me during the work with this thesis. I especially wish to express my gratitude:

to my teacher in research Professor Börje Johansson for his encouragement and unfailing readiness to discuss and to help in all possible ways. It has been a privilege to work with him,

to my collaborator and friend Dr Bengt Uvelius,

to my colleagues in the laboratory Drs Per Hellstrand and Anders Arner,

to the technical assistants Ina Nordström, Monica Heidenholm and Monica Lundahl,

to Monica Lundahl and Kristina Borglid for skilled typing of this and other manuscripts,

to Monica Heidenholm for skilfully preparing the line drawings of this and other manuscripts,

to Docent Wolfgang Grampp for his valuable help and discussions,

to Docent Hans Örnhammar for valuable discussions and friendly support,

to Professor Johann Axelsson who first introduced me into the field of physiology,

to Civ.ing. Lars Sjölin and to the staff in the department workshop headed by Leif Nilsson,

to Olle hammar for his help in preparing illustrations and solving all kinds of problems.

This study was supported by the Swedish Medical Research Council (04X-00028), the Medical Faculty, University of Lund and AB Hässle, Göteborg.

REFERENCES

- Andersson, C., Hellstrand, P., Johansson, B. and Ringberg, A. 1974.
Contraction in venous smooth muscle induced by hypertonicity. Calcium dependence and mechanical characteristics. *Acta Physiol Scand* 90:451-461.
- Arvill, A., Johansson, B. and Jonsson, O. 1969. Effects of hyperosmolarity on the volume of vascular smooth muscle cells and relation between cell volume and muscle activity. *Acta Physiol Scand* 75:484-495.
- Basar, E. and Weiss, C. 1969. Rate sensitivity of the mechanism of pressure induced change of vascular resistance. *Kybernetik* 5:241-247.
- Bayliss, W.M. 1902. On the local reactions of the arterial wall to changes in internal pressure. *J. Physiol.* 28:220-231.
- Bennet, M.R. and Burnstock G. 1966. Application of the sucrose-gap method to determine the ionic basis of the membrane potential of smooth muscle. *J. Physiol.* 183:637-648.
- Biamino, G. and Johansson B. 1970. Effects of calcium and sodium on contracture tension in the smooth muscle of the rat portal vein. *Pflügers. Arch. ges. Physiol.* 321:143-158.
- Blaustein, M.P. 1977. Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. *Am. J. Physiol.* 232(3):C165-C173.
- Bohr, D.F. 1974. Reactivity of vascular smooth muscle from normal and hypertensive rats: Effect of several cations. *Fed. Proc.* 33:127-132.
- Bohr, D.F. 1978. Vascular smooth muscle. In: *The peripheral circulation.* Edited by P.C. Johnson. New York:Wiley p.13-43.
- Bülbring, E. and Tomita T. 1970. Calcium and the action potential in smooth muscle. In: *Calcium and cellular function.* Edited by A.W. Cuthbert. Macmillan. p.249-260.
- Bülbring, E. and Tomita, T. 1977. Calcium requirement for the α -action of catecholamines on guinea-pig taenia coli. *Proc. R. Soc. London ser B.* 197:271-284.

- Carrier, O., Jr., Walker, J.R. and Guyton, A.C. 1964. Role of oxygen in autoregulation of blood flow in isolated vessels. *Am. J. Physiol.* 206:951-954.
- Casteels, R. and Wuytack, F. 1975. Aerobic and anaerobic metabolism in smooth muscle cells of taenia coli in relation to active ion transport. *J. Physiol.* 250:203-220.
- Collins, G.A., Sutter, M.C. and Teiser, J.C. 1972a. Calcium and contraction in the rabbit anterior mesenteric portal vein. *Canad. J. Physiol. Pharmacol.* 50:289-299.
- Collins, G.A., Sutter, M.C. and Teiser, J.C. 1972b. The effect of manganese on the rabbit anterior-mesenteric portal vein. *Canad. J. Physiol. Pharmacol.* 50:300-309.
- Detar, R. and Bohr, D.F. 1968. Oxygen and vascular smooth muscle contraction. *Am. J. Physiol.* 214:241-244.
- Detar, R. and Bohr, D.F. 1972. Contractile responses of isolated vascular smooth muscle during prolonged exposure to anoxia. *Am. J. Physiol.* 222:1269-1277.
- Devine, C.E., Somlyo, A.V. and Somlyo, A.P. 1973. Sarcoplasmic reticulum and mitochondria as cation accumulation sites in smooth muscle. *Phil. Trans. R. Soc. Lond. B.* 265:17-23.
- Droogmans, G., Raeymaekers, L. and Casteels, R. 1977. Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.* 70:129-148.
- Droogmans, G. and Casteels, R. 1979. Sodium and calcium interactions in vascular smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.* 74:57-70.
- Ebashi, S. 1979. Ca ion and muscle contraction. In: *Proc 7th Int. Congr. Pharmacol.* 3: 81-98.
- Ebashi, S. and Endo, M. 1968. Calcium ions and muscle activation. *Prog. Biophys. Mol. Biol.* 18:123-185.

- Endo, M., Kitazawa, T., Yagi, S., Iino, M. and Kakuta, Y. 1977. Some properties of chemically skinned smooth muscle fibres. In: Excitation-contraction coupling in smooth muscle. Edited by R. Casteels, T. Goodfraid and J. Rüegg. Amsterdam: Elsevier. p199-209.
- Fairchild, H.M., Ross, J.M. and Guyton, A.C. 1966. Failure of recovery from reactive hyperemia in the absence of oxygen. *Am. J. Physiol.* 210:490-492..
- Fay, F.S., Nair, P. and Whalen, W.J. 1977. Mechanism of oxygen induced contraction of ductus arteriosus. In: Tissue hypoxia and ischemia. Edited by M. Reivich, R. Coburn, S. Lahiri and B. Chance. New York, Plenum. p123-134.
- Folkow, B. 1962. Transmural pressure and vascular tone - some aspects of an old controversy. *Arch. Int. Pharmacodyn.* 139:445-469.
- Folkow, B. 1964. Description of the myogenic hypothesis. *Circulat. Res.* 15:suppl I. p 279-285.
- Fozzard, H.A. 1977. Heart: excitation-contraction coupling. *Ann. Rev. Physiol.* 39:201-220.
- Golenhofen, K., Hermstein, N. and Lammel, E. 1973. Membrane potential and contraction of smooth muscle (portal vein) during application of noradrenaline and high potassium and selective inhibitory effects of iproveratril (verapamil). *Microvascular Res.* 5:73-80.
- Gordon, A.R. 1978. Contraction of detergent-treated smooth muscle. *Proc. Natl. Acad. Sci. USA.* 75:3527-3530.
- Grände, P.-O., Lundvall, J. and Mellander, S. 1977. Evidence for a rate-sensitive regulatory mechanism in myogenic microvascular control. *Acta Physiol Scand* 99:432-447.
- Grände, P.-O. 1979. Dynamic and static components in the myogenic control of vascular tone in cat skeletal muscle. *Acta Physiol Scand Suppl* 476.
- Guyton, A.C., Ross, J.M., Carrier, O. Jr. and Walker, J.R. 1964. Evidence for tissue oxygen demand as the major factor causing autoregulation. *Circulat. Res. Suppl. I.* 60-68.

- Grün, A. and Fleckenstein, A. 1972. Die elektromechanische Entkopplung der glatten Gefäßmuskulatur als Grundprinzip der Coronardilatation durch 4-(2-Nitrophenyl)-2, 6-dimethyl-1,4 dihydropipidin-3,5 dicarbonsäure-dimethylester (Bay a 1040 Nifedipine) Drug. Res. 22:334-344.
- Haljamäe, H., Johansson, B., Jonsson, O. and Röckert, H. 1970. The distribution of sodium, potassium and chloride in the smooth muscle of the rat portal vein. Acta Physiol Scand 78:255-268.
- Harris, E.J. 1960. Transport and accumulation in biological system. p.2. Butterworths scientific publication London.
- Hellstrand, P., Johansson, B. and Ringberg, A. 1972. Influence of extracellular calcium on isometric force and velocity of shortening in depolarized venous smooth muscle. Acta Physiol Scand 84:528-537.
- Hellstrand, P. 1977. Oxygen consumption and lactate production of the rat portal vein in relation to its contractile activity. Acta Physiol Scand 100:91-106.
- Hellstrand, P., Johansson, B. and Norberg, K. 1977. Mechanical, electrical and biochemical effects of hypoxia and substrate removal on spontaneously active vascular smooth muscle. Acta Physiol Scand 100:69-83.
- Hellstrand, P. 1978. Effects of hypoxia on the rat portal vein in vitro: P_{O_2} gradients in tissue and surrounding fluid. Acta Physiol Scand 103:472-474.
- Hellstrand, P. (1979). Mechanical and metabolic properties related to contraction in smooth muscle. Acta Physiol Scand. suppl. 464
- Hellstrand, P. and Arner, A. 1980. Contraction of the rat portal vein in hypertonic and isotonic medium: mechanical properties and effects of Mg^{2+} . Acta Physiol Scand 000:000-000.
- Hermesmeyer, K. 1976. Ba^{2+} and K^{+} alteration of K^{+} -conductance in spontaneously active vascular smooth muscle. Am. J. Physiol. 230:1031-1036.
- Hubbard, J.I., Jones, S.F. and Landau, E.M. 1958. On the mechanism by which calcium and magnesium affect the spontaneous release of transmitter from mammalian motor nerve terminals. J. Physiol. 194:355-380.

- Hudgin, P.M. and Weiss, G.B. 1969. Effects of Ba, Sr and stimulatory agents on Ca movements and contraction in vascular smooth muscle. Fed. Proc. 28:541.
- Huxley, A.F. 1974. Review lecture: Muscular contraction. J. Physiol. 243:1-43.
- Ito, Y. and Kuriyama, H. 1971. Membrane properties of the smooth muscle fibres of the guinea-pig portal vein. J. Physiol. 214:427-441.
- Johansson, B. and Jonsson, O. 1968. Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. Acta Physiol Scand 72:456-468.
- Johansson, B. 1971. Electromechanical and mechanoelectrical coupling in vascular smooth muscle. Angiologica 8:129-143.
- Johansson, B. and Mellander, S. 1975. Static and dynamic components in the vascular myogenic response to passive changes in length as revealed by electrical and mechanical recordings from the rat portal vein. Circulat. Res. 36:76-83.
- Johansson, B. and Somlyo, A.P. 1980. Electrophysiology and excitation-contraction coupling. In: Handbook of physiology - The cardiovascular system II. In press.
- Johnson, P.C. (ed) 1964. Symposium on autoregulation of blood flow. Circulat. Res. 15:suppl.I 1-291.
- Johnson, P.C. 1974. The microcirculation and local humoral control of the circulation. In: MTP International review of science. Cardiovascular Physiology I. Edited by Guyton and Jones. Butterworth, London. University Park Press, Baltimore.
- Jonsson, O. 1970. Extracellular osmolality and vascular smooth muscle activity. Acta Physiol Scand suppl 359.
- Keatinge, W.R. 1968. Ionic requirements for arterial action potential. J. Physiol. 194:169-182.
- Kohlhardt, M., Haastert, H.P. and Krause, H. 1973. Evidence of non-specificity of the Ca-channel in mammalian myocardial fibre membranes. Pflügers Arch. ges Physiol. 342:125-136.

- Linke, A.M., Knehr, H. and Betz, E. 1979. The influence of extracellular pH on the contractile behaviour and the NAD/NADH-fluorescence of isolated spontaneously active portal veins. *Pflügers Arch.* 382: suppl.R26.
- Litten, R.Z., Solaro, R.J. and Ford, G.D. 1977. Properties of the calcium-sensitive components of bovine arterial actomyosin. *Arch. Biochem. Biophys.* 182:24-32.
- Ljung, B. 1970. Nervous and myogenic mechanism in the control of a vascular neuroeffector system. *Acta Physiol Scand suppl.* 349. p.33-68.
- Lundvall, J. 1972. Tissue hyperosmolality as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle. *Acta Physiol Scand suppl.* 379.
- McKinley, M.J., McKenzie, J.S. and Blair-West, J.R. 1974. Effects of maintained osmolarity changes on rat portal vein spontaneous contractions. *Am. J. Physiol.* 226: 475-494.
- Mellander, S., Johansson, B., Gray, S., Jonsson, O., Lundvall, J. and Ljung, B. 1967. The effects of hyperosmolality on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. *Angiologica* 4:310-322.
- Mellander, S. and Johansson, B. 1968. Control of resistance, exchange and capacitance functions in the peripheral circulation. *Pharmacol Rev.* 20:117-196.
- Mellander, S., Grände, P.-O. and Borgström, P. 1980. Static and dynamic components in myogenic vascular response. In: *Vascular neuroeffector mechanism*. Edited by J.A. Bevan, T. Godfraind, R.A. Maxwell and P.M. Vanhoutte. New York. Raven Press. p.199-206.
- Mrwa, U., Achtig, I. and Rüegg, J.C. 1974. Influences of calcium concentration and pH on the tension development and ATPase activity of the arterial actomyosin contractile system. *Blood Vessels* 11:277-286.
- Murphy, R.A., Bohr, D.F. and Newman, D.L. 1969. Arterial actomyosin: Mg, Ca and ATP ion dependencies for ATPase activity. *Am. J. Physiol.* 217:666-673.

- Namm, D.H. and Zucker, J.L. 1973. Biochemical alterations caused by hypoxia in the isolated rabbit aorta. *Circulat. Res.* 32:464-470.
- Palaty, V. 1974. Regulation of the cell magnesium in vascular smooth muscle. *J. Physiol.* 242:555-569.
- Peiper, U., Griebel, L. and Wende, W. 1971. Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: Two different mechanisms. *Pflügers Arch. ges Physiol.* 330:74-89.
- Paul, R.J. and Rüegg, J.C. 1978. Biochemistry of vascular smooth muscle: Energy metabolism and proteins of the contractile apparatus. In: *Micro-circulation Vol II* (ed. G. Kaley and B.M. Altura), p 41-82. University Park Press, Baltimore.
- Paul, R.J., Bauer, M. and Pease, W. 1979. Vascular smooth muscle: Aerobic glycolysis linked to sodium and potassium transport processes. *Science* 206:1414-1416.
- Pittman, R. N. and Duling, B.R. 1973. Oxygen sensitivity of vascular smooth muscle I. In vitro studies. *Microvasc. Res.* 6:202-211.
- Reuter, H., Blaustein, M.P. and Haeusler, G. 1973. Na-Ca exchange and tension development in arterial smooth muscle. *Phil Trans. R. Soc. Lond. B.* 265:87-94.
- Small, J.V. and Sobieszek A. 1977. Myosin phosphorylation and Ca-regulation in vertebrate smooth muscle. In: *Excitation contraction coupling in smooth muscle*. Edited by R. Casteels, T. Godfraind and J.C. Rüegg. Elsevier/North Holland p.385-393.
- Somlyo, A.V. and Somlyo, A.P. 1968. Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmacol. Exptl. Therap.* 159:129-145.
- Somlyo, A.P. and Somlyo, A.V. 1971. Strontium accumulation by sarcoplasmic reticulum and mitochondria in vascular smooth muscle. *Science* 174:955-958.
- Somlyo, A.P., Somlyo, A.V., Devine, C.E., Peters, P.D. and Hall, T.A. 1974. Electron microscopy and electron probe analysis of mitochondrial cation accumulation in smooth muscle. *J. Cell. Biol.* 61:723-742.

- Somlyo, A.P., Somlyo, A.V. and Shuman, H. 1979. Electron probe analysis of vascular smooth muscle: composition of mitochondria nuclei and cytoplasm. *J. Cell. Biol.* 80:316-335.
- Sparrow, M.P., Maxwell, L.C., Rüegg, J.C. and Bohr, D.F. 1970. Preparation and properties of a calcium ion-sensitive actomyosin from arteries. *Am. J. Physiol.* 219:1366-1372.
- Teorell, T. 1971. A biophysical analysis of mechano-electrical transduction. In: *Handbook of sensory physiology 1*. Edited by Loewenstein. p.295. Springer Verlag. Berlin.
- Uvelius, B. and Sigurdsson, S.B. 1980. Effects of Sr^{2+} and some other divalent cations on electrical and mechanical activity in rat portal vein. In: *Handbook of stable strontium*. Plenum Publ. corp. In press.
- Wahlström, B. 1971. The effects of changes in the ionic environment on venous smooth muscle distribution of sodium and potassium. *Acta Physiol Scand* 82:382-392.
- Wahlström, B.A. 1973. A study of the action of noradrenaline on ionic content and sodium, potassium and chloride effluxes in the rat portal vein. *Acta Physiol Scand* 89:522-530.
- van Bremen, C., Farinas, B.R., Gerba, P. and McNaughton, E.D. 1972. Contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium efflux. *Circulat. Res.* 30:44-54.
- van Bremen, C. 1976. Transmembrane calcium transport in vascular smooth muscle. In: *Vascular neuroeffector mechanism 2nd Int. Symp.* Odense. Basel, Karger p.67-79.
- Wei, J.-W., Janis, R.A. and Daniel, E.E. 1977. Alterations in calcium transport and binding by the plasma membrane of mesenteric arteries from spontaneously hypertensive rats. *Blood Vessels* 14:55-64.
- Weiss, G.B. 1977. Calcium and contractility in vascular smooth muscle. In: *Advances in general and cellular pharmacology. Vol II* edited by Narahashi and Beanchi. Plenum Press p. 71-153.
- Yeh, J.Z., Oxford, G.S., Wu, C.H. and Narahashi, T. 1976. Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys J.* 16:77.

